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Magnesium injury of wheat

SAM F. TRELEASE AND HELEN M. TRELEASE

(WITH TWO TEXT FIGURES AND PLATES 5–8)

A pronounced pathological modification of leaves of young wheat plants growing in culture solutions was described by Tottingham (1914) and termed by him magnesium injury. The most characteristic form of this leaf injury is represented by a rolling and coiling of the young leaf during emergence from the sheath. The symptoms of magnesium injury of wheat are so conspicuous and distinctive that this disease furnishes a remarkable example of growth derangement resulting from disturbed salt nutrition. This injury was described by Tottingham (1914), Shive (1915), and Trelease (1920) as characteristic of cultures whose solutions had high ratios of magnesium sulphate to calcium nitrate. The occurrence and severity of magnesium injury appear to be definitely related to the ionic ratio of magnesium to calcium in the culture solution. The production of this injury is one of the most striking cases in which a clear relation has been demonstrated between an ionic ratio and the development of the plant.

The experiments reported in the present paper were undertaken with the aim of obtaining further information concerning the nature and symptoms of magnesium injury of wheat and the conditions determining its appearance in plants grown in solution cultures. In particular, this study was planned to include tests that would indicate more clearly than previous work whether the characteristic symptoms are to be ascribed to magnesium toxicity or to calcium deficiency.

GENERAL METHODS

The culture methods employed in this study were essentially the same as those described in previous papers (Trelease and Livingston, 1924; Trelease and Trelease, 1924, 1928). Marquis spring wheat (supplied by the University of Saskatchewan) was soaked for three hours in distilled water and then germinated on wet filter paper in glass culture dishes (24 cm. in diameter). When the primary root of each seedling was about 6 mm. long, selected seedlings were placed upon paraffined bobbinet stretched over enamelled iron pans (25 cm. in diameter and 8 cm. deep) filled with distilled water. Each of the pans was placed in a similar, but slightly larger pan, and distilled water was poured into the space between the two

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TABLE 1

Magnesium injury and mean dry weights of tops of wheat plants of series 1, grown for 49 days, from March 22 to May 10, 1929, in culture solutions varying in ratio of magnesium chloride to calcium chloride. Actual concentrations of magnesium chloride and calcium chloride in these solutions are four times as great as those for series 2.[a]

SOLUTION NUMBER	Mg/Ca RATIO	CONCENTRATION OF $MgCl_2$	CONCENTRATION OF $CaCl_2$	MAGNESIUM INJURY OF THIRD LEAF		MEAN DRY WEIGHTS	
				Number	Percentage	Actual	Relative
		M	*M*			*Milligrams*	
1	1.00	0.01	0.01000	0	0	125.0±1.9	100
2	1.75	0.01	0.00572	0	0	120.4±2.6	96
3	3.06	0.01	0.00327	0	0	116.5±1.9	93
4	5.35	0.01	0.00187	0	0	104.1±1.5	83
5	9.35	0.01	0.00107	12	30	89.3±1.6	71
6	16.39	0.01	0.00061	32	80	76.3±1.2	61
7	28.57	0.01	0.00035	37	93	62.3±1.4	50
8	50.00	0.01	0.00020	40	100	48.4±1.0	39

[a] Each solution also contained 0.002 M KH_2PO_4, 0.002 M K_2SO_4, 0.002 M NH_4NO_3, and 0.00001 M $FeSO_4$.

pans until the level of the water was even with the top of the smaller pan. During the first two days a moist chamber was provided for the seedlings by placing the cover of a glass culture dish on each netting. On the next day selected seedlings about 4 cm. high were transferred to the culture solutions.

The culture vessels were cylindrical, glazed earthenware jars (so-called chemical stoneware) obtained from the General Ceramics Company. Each jar had a capacity of about 7000 cc. For use as a culture vessel, the jar was nearly filled with solution and covered with a paraffined Portland cement disk having five circular openings, in which were set the cork stoppers supporting the plants (pl. 8). Each stopper bore eight seedlings, thus making forty seedlings in a culture. The cultures were placed on rotating tables in the greenhouse where all of the plants were exposed to approximately the same fluctuations in environmental conditions throughout the period of the experiment.

The solutions were renewed frequently enough for each plant to be supplied with an amount of solution corresponding to about 56 cc. per day. The seedlings were grown in the culture solutions for 40 or 49 days. Notes were made of the appearance of the tops and roots, special attention being given to symptoms of magnesium injury. At the conclusion of the culture period the tops of the individual plants of each culture were harvested separately and dried for 20 hours, to an approximately constant weight, at 101° C. The top dry weight of each plant was then determined.

Four series of cultures are described in the present paper. Data for these are shown in tables 1–4, respectively. Each table gives the period of the experiment and the culture solutions employed. It also shows the data that were obtained for magnesium injury and dry weights of tops. The first series was carried on in one of the old greenhouses of Columbia University; the last three series were conducted simultaneously in the new roof greenhouse, which forms the twelfth story of Schermerhorn Extension.

SYMPTOMS OF MAGNESIUM INJURY

Injury of the third leaf

Emergence of tightly rolled leaf apex. Magnesium injury becomes visible after young wheat plants have grown in the culture solutions for from nine to twelve days, when the young, third leaf is just emerging from the sheath. The coleoptile and the first and second green leaves develop normally. But the third leaf of some cultures shows characteristic symptoms of injury. As it emerges from the sheath, the injured leaf is much more tightly rolled than a normal leaf. The rolling of the leaves is in the same

TABLE 2

Magnesium injury and mean dry weights of tops of wheat plants of series 2, grown for 40 days, from October 31 to December 10, 1930, in culture solutions varying in the ratio of magnesium chloride to calcium chloride. Actual concentrations of magnesium chloride and calcium chloride in these solutions are one-fourth as great as those for series 1.[a]

SOLUTION NUMBER	Mg/Ca RATIO	CONCENTRATION OF $MgCl_2$	CONCENTRATION OF $CaCl_2$	MAGNESIUM INJURY				MEAN DRY WEIGHTS	
				Third leaf		Fourth leaf			
				Number	Percentage	Number	Percentage	Actual	Relative
		M	*M*					*Milligrams*	
1	1.00	0.0025	0.002500	0	0	1	3	115.1±2.1	100
2	1.75	0.0025	0.001429	0	0	7	18	108.2±2.2	94
3	3.06	0.0025	0.000817	1	3	31	78	101.3±2.2	88
4	5.35	0.0025	0.000467	2	5	39	98	94.6±1.9	82
5	9.35	0.0025	0.000267	13	33	40	100	82.3±1.4	72
6	16.39	0.0025	0.000153	37	93	40[b]	100	70.1±1.0	61
7	28.57	0.0025	0.000088	40	100	40[c]	100	67.3±0.7	58
8	50.00	0.0025	0.000050	40	100	40[d]	100	62.0±0.7	54

[a] Each solution also contained 0.002 M KH_2PO_4, 0.002 M K_2SO_4, 0.002 M NH_4NO_3, and 0.00001 M $FeSO_4$.

[b] Only 29 fourth leaves had emerged from sheath.

[c] Only 9 fourth leaves had emerged from sheath.

[d] No fourth leaves had emerged from sheath.

direction as that of the normal young leaf—that is, the upper surface of the blade remains inside (fig. 4, pl. 6; fig. 9, 10, 11, pl. 7). The injured leaf is darker green in color than a healthy leaf, and it grows out of the sheath at a slower rate (fig. 2, pl. 5). The apical portion that has emerged does not unroll subsequently; it dries and remains as a stiff, wire-like structure, that may be erect or sinuous.

Spiral deformation within sheath. The most striking and characteristic abnormality of a leaf affected with magnesium injury is a spiral deformation that develops below the tightly rolled apical portion (fig. 3, pl. 5; fig. 4, 5, 6, pl. 6; fig. 8, pl. 7). The spiral forms within the sheath while the needle-like apical portion of the leaf is emerging. The spiral develops as a result of the failure of the apical part to move upward through the sheath as rapidly as elongation occurs at the base of the leaf. A wilting or withering of the leaf tissues appears to occur in the region of the spiral, and continued growth pressure from below crushes a considerable length of the enclosed leaf into a close spiral, which may vary in length from a few millimeters to a centimeter or more. The upper part of the surrounding sheath seems to be rolled so tightly that it obstructs the upward movement of the distal portion of the leaf. The spiral that is produced does not wind continuously in one direction; the direction usually is reversed several times, with compact, irregular loops separating the more perfectly formed spiral regions.

The mechanical process by which the spiral is formed may be simulated by forcing a considerable length of wire into a glass tube, closed at the far end and having a bore several times the diameter of the wire. The resulting distortion of the wire, into a close spiral with several reversals of direction, appears to be essentially the same as that of the leaf. Pressure from below, rather than a contraction within the region of the spiral, results in the coiling. Yet a spiral that has been lengthened by application of tension contracts partially when the tension is released.

Severely injured portions of the leaf exhibit a dark-green color that suggests an infiltration of intercellular spaces with liquid. The compressed, dark-green spiral may be seen as a silhouette within the translucent sheath if the plant is viewed by transmitted sunlight. This may be seen with special clearness when an injured fourth leaf is contained within the sheath of a normally developed third leaf.

Emergence of spiral from sheath. In cases of very severe injury, growth of the injured leaf soon ceases, so that only the very tightly rolled, dark-green apex of the leaf emerges from the sheath (fig. 2, pl. 5). But if injury is less severe, basal growth of the leaf continues for some time and the coiled part of the leaf emerges from the sheath (fig. 3, pl. 5), followed by a rolled portion below the spiral. The spiral generally assumes a horizontal

TABLE 3

Magnesium injury and mean dry weights of tops of wheat plants of series 3, grown for 40 days, from October 31 to December 10, 1930, in culture solutions varying in the ratio of magnesium chloride to calcium chloride. Solution 1 of this series is the same as solution 3 of series 1.[a]

SOLUTION NUMBER	Mg/Ca RATIO	CONCENTRATION OF $MgCl_2$	CONCENTRATION OF $CaCl_2$	MAGNESIUM INJURY				MEAN DRY WEIGHTS	
				Third leaf		Fourth leaf			
				Number	Percentage	Number	Percentage	Actual	Relative
		M	*M*					*Milligrams*	
1	3.06	0.0100	0.00327	1	3	25	63	120.4±2.5	100
2	5.35	0.0175	0.00327	2	5	32	80	105.5±2.2	88
3	9.35	0.0306	0.00327	4	10	34	85	104.5±2.0	87
4	16.39	0.0536	0.00327	10	25	33	83	86.5±1.4	72

[a] Each solution also contained 0.002 M KH_2PO_4, 0.002 M K_2SO_4, 0.002 M NH_4NO_3, and 0.00001 M $FeSO_4$.

TABLE 4

Magnesium injury and mean dry weights of tops of wheat plants of series 4, grown for 40 days, from October 31 to December 10, 1930, in culture solutions varying in the concentration of strontium chloride. Solution 1 of this series is the same as solution 6 of series 1.[a]

SOLUTION NUMBER	CONCENTRATION OF $SrCl_2$	Mg/(Ca+Sr) RATIO	MAGNESIUM INJURY				MEAN DRY WEIGHTS	
			Third leaf		Fourth leaf			
			Number	Percentage	Number	Percentage	Actual	Relative
	M						*Milligrams*	
1	0.00000	16.39	34	85	40	100	76.1±1.2	100
2	0.00001	16.13	33	83	37	93	84.3±1.4	111
3	0.00003	15.63	32	80	33	83	86.3±1.6	113
4	0.00010	14.08	26	65	35	88	87.6±1.3	115
5	0.00032	10.75	20	50	32	80	89.2±1.5	117
6	0.00100	6.21	21	53	33	83	95.2±1.6	125
7	0.00316	2.65	13	33	—[b]	—	75.6±1.5	99
8	0.01000	0.94	1	3	—[c]	—	40.7±0.4	53

[a] Each solution also contained 0.002 M KH_2PO_4, 0.002 M K_2SO_4, 0.002 M NH_4NO_3, 0.00001 M $FeSO_4$, 0.010 M $MgCl_2$, and 0.00061 M $CaCl_2$. Ratio of $MgCl_2$ to $CaCl_2$, 16.39.

[b] Only 7 fourth leaves had emerged from sheath.

[c] Only 4 fourth leaves had emerged from sheath.

or drooping position after emergence (fig. 4, 5, pl. 6; fig. 8, pl. 7). Rarely, a second spiral forms within the sheath and later emerges (fig. 6, pl. 6).

Emergence of lower portion of leaf. As just mentioned, if the injury is very severe, only the needle-like apical portion, from one to five centimeters in length, emerges from the sheath. In rare cases emergence of the lower part of the leaf occurs through the side of the enveloping sheath rather than at the top (fig. 7, pl. 6). The apex of the leaf may first emerge for a short distance out of the opening at the top of the sheath. Then a part of the leaf several centimeters long wilts, becomes very thin and thread-like, and remains irregularly coiled and looped within the sheath. Finally a region below this remains turgid and, though greatly distorted, is forced out through the side of the sheath, unrolling the latter sufficiently to permit a considerable portion of the leaf to emerge.

If the injury is somewhat less severe, the spiral issues at the top of the sheath, followed by a tightly rolled portion that does not expand after emergence. When the injury is milder, the part of the leaf below the spiral emerges rapidly and expands normally; at maturity the leaf attains the same length as that of an uninjured leaf, twenty to twenty-five centimeters (fig. 9, pl. 7). A still lower degree of injury is characterized by the formation of a long, loose spiral, several centimeters in length, followed by a normally expanded blade (fig. 10, pl. 7).

In other cases no spiral is formed. Below the tightly rolled apex emergence is rapid, and the only sign of injury in the lower portion is the presence in the expanded leaf of one or more marginal lacerations, sometimes extending to the midrib (note laceration in fig. 10, pl. 7). Very slight injury of many leaves is indicated by a tightly rolled apical portion, often from one to three centimeters in length (fig. 11, pl. 7). The tip of the leaf is closely rolled as it emerges from the sheath and it fails to expand later. But the rest of the leaf emerges and unrolls normally and reaches the usual size of healthy leaves. This type of slight injury is frequently observed in rapidly growing plants, and it has only slight influence on final dry yields. Leaf injury apparently never occurs in leaves that have emerged normally and have developed to the usual size. Old leaves frequently exhibit rolled apices, which remain green for some time and finally die and become yellow. But apical injury of these leaves appears always to have occurred while the leaf was in an early stage of its development and emerging from the sheath.

Leaf-tip abscission. Abscission of the tightly rolled, needle-like tips of injured leaves frequently occurs. In the most severely injured plants leaf-tip abscission may take place after the seedlings have been in the culture solutions for only ten days. The tip, two or three centimeters long, then

[b] Only 7 fourth leaves had emerged from sheath.
[a] Only 4 fourth leaves had emerged from sheath.

breaks from the plant at the sheath opening (position clearly shown in fig. 2, pl. 5); subsequent examination of the plant may reveal no external sign of the injured leaf. Most of the leaf tip is dark-green in color when it becomes separated from the plant; a yellow discoloration usually extends only a very short distance above the point of abscission. When the injury is less severe, abscission may occur later, at the base of the spiral after that has emerged. In cases of very slight injury the rolled apex frequently becomes yellow and dry, but it usually remains attached to the rest of the leaf (fig. 11, pl. 7).

Injury of the fourth leaf

The symptoms of magnesium injury of the fourth leaf are similar to those just described for the third leaf. Injury of the fourth leaf tends to be of more general occurrence, however. In many cases the fourth leaf is affected even though the third leaf has escaped injury. Thus, eighty per cent of the fourth leaves of a culture may be affected after all but two per cent of the third leaves have escaped injury. If the third leaf has been very severely injured, the fourth leaf usually fails entirely to emerge from the sheath, though in rare cases emergence may occur at a lower point than normal, through the side of the sheath. The fifth leaf may exhibit the typical symptoms of magnesium injury even though earlier leaves have developed normally.

Other derangements accompanying magnesium injury of leaves

Dark-green color of leaves of injured plants. Leaves of plants suffering from magnesium injury are greener in color than those of normal plants. This suggests a direct relation between the color and the amount of magnesium present in the cells. The greenness of the foliage is roughly proportional to the severity of the magnesium injury; the color of the foliage is darkest in the most severely injured plants, and the depth of green decreases in passing from severely to slightly injured plants. The apical parts of the severely injured leaves generally die and then become yellow or brown, but the living parts remain dark green in color.

Premature tillering associated with leaf injury. A number of tillers develop prematurely on plants exhibiting the most pronounced abnormalities of leaf development, and the tillers themselves show characteristic symptoms of magnesium injury. These tillers usually arise at the time when the third or fourth leaf is appearing on normal plants, and they develop in greatest numbers on plants which are so severely injured that the third or fourth leaf fails to emerge fully from the sheath. This suggests that such premature branching from the base of the stem is a response to severe injury of the stem apex, resulting in loss of apical dominance.

Anatomical and histological studies would be of great interest in this connection. Premature tillering of course is not a specific symptom of magnesium injury. It is very conspicuous, for example, in plants suffering from strontium poisoning. The tillers that develop as a result of magnesium toxicity generally exhibit the characteristic symptoms of magnesium injury (fig. 1, pl. 5).

Inhibition of root development. Root development may be markedly retarded in plants exhibiting symptoms of the most pronounced leaf injury. Growth in length, both of the main roots and of their lateral branches, proceeds less rapidly and ceases earlier than in the less severely injured plants. Thus, a stunted root system is associated with severe magnesium injury of the foliage. Root development is apparently normal, however, if injury to the tops is slight.

Production of adventitious roots. Extremely severe magnesium injury of the primary root of a wheat seedling often results in the early development of several adventitious roots. Six roots, instead of the usual two or four, frequently push out from the young seedling at about the same level as the central primary root, and then still another adventitious root may emerge very early from the stem, just above the primary root. These adventitious roots seem to develop prematurely as a response to severe injury of the primary root. They appear to be less sensitive to the toxic medium than the primary root and the first lateral pair of seminal roots, since they frequently grow to a much greater length in the solution. This suggests the possibility that these roots are less permeable to the toxic substance. The development of several adventitious roots of this kind, however, is not a specific symptom of magnesium injury; it may result also when other poisons—copper salts, for example—are supplied in highly toxic concentrations.

CONDITIONS INFLUENCING MAGNESIUM INJURY

Magnesium injury in relation to the ratio of magnesium to calcium in the culture solution

The occurrence of the characteristic form of leaf injury described in the preceding section of this paper has been shown to depend upon the ionic ratio of magnesium to calcium in the culture solution (Tottingham, 1914; Shive, 1915; Trelease, 1920). This is one of the few cases thus far reported in which a clear relation has been demonstrated between the value of an ionic ratio and the development of the plant.

Tottingham (1914) employed eighty-four solutions containing KNO_3, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ in each of three series of wheat cultures. All possible proportions of the four salts were used that could be produced

by varying each salt by one-tenth of the total osmotic concentration of all salts. The three series had concentrations corresponding to 0.05, 2.50, and 8.15 atmospheres, respectively. No leaf injury was observed in the cultures of the first series. But the characteristic rolling and coiling of the young leaves, termed magnesium injury by Tottingham, was conspicuous in the second and third series, having the higher concentrations. Magnesium injury was clearly related in these series to the ratio of magnesium to calcium in the solutions. The cultures characterized by the highest values of this ratio were all severely injured, those with the lowest values were free from injury, and those with intermediate values showed some injury. No

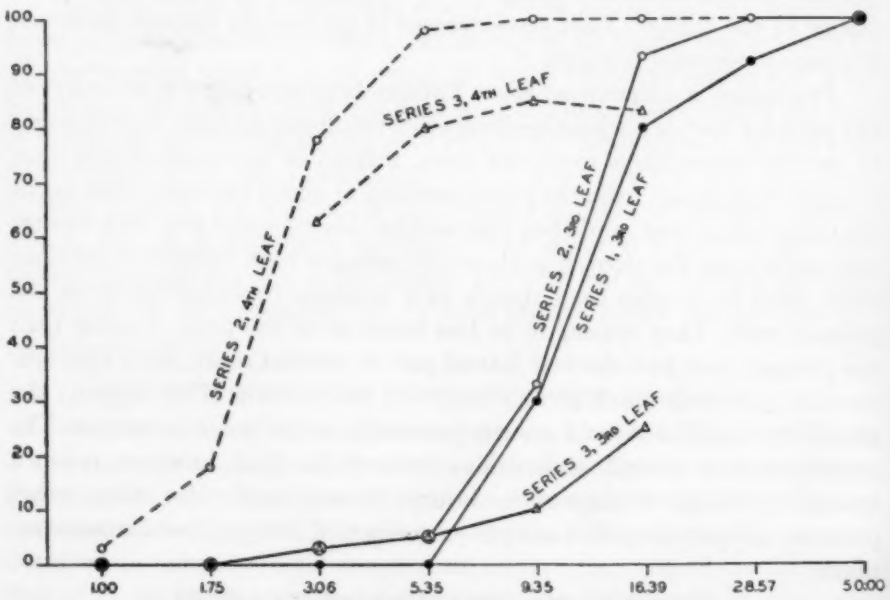

Fig. 1. Magnesium injury of wheat plants as related to the magnesium-calcium ratios of the culture solutions. Abscissas represent magnesium-calcium ratio values plotted on a logarithmic scale. Ordinates represent percentages of leaves exhibiting magnesium injury.

Graphs for series 1–3 are plotted from the data of tables 1–3.

injury occurred with ratio values below 0.40, when the osmotic value was 2.50 atmospheres, and none appeared below 0.28, when the osmotic value was 8.15 atmospheres.

Shive (1915) found that when his three-salt solutions, containing KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$, had an osmotic value of 1.75 atmospheres and magnesium-calcium ratios of less than 1.5, the plants were free from magnesium injury, and that when the solutions had an osmotic value of

4.00 atmospheres this limit occurred with the ratio value 2.2. Shive demonstrated clearly that the occurrence of injury was set by the magnitude of the ratio of magnesium to calcium. It was not determined by the actual concentration of any one salt or ion, nor did it bear any definite relation to either of the ratios Mg/K or Ca/K.

Trelease (1920) used eighty-four solutions like those of one of Tottingham's series but with KNO_3 replaced by KCl. With these solutions having an osmotic value of 1.60 atmospheres, severe magnesium injury was clearly confined to ratio values that lay between 1.65 and 13.43 (the highest tested); slight injury occurred when the magnesium-calcium ratios lay between the limits 0.81 and 3.64; and entire freedom from this form of injury was shown in the cultures which had ratio values below 0.81.

Severity of injury as related to the magnesium-calcium ratio. Series 1–3 of the present study bring out a very clear relation between the severity of magnesium injury and the ratio of magnesium to calcium in the culture solution. The results are presented in tables 1–3 and they are plotted in figure 1, in which the abscissas represent the ratios of magnesium to calcium (plotted on a logarithmic scale) and the ordinates represent the percentages of leaves exhibiting magnesium injury.

The graphs of figure 1 show that a marked rise in the percentage of injured leaves accompanied an increase in the value of the ratio of magnesium to calcium. They also show that injury to fourth leaves was much more general than to third leaves. It is interesting to note that series 1 and 2 gave essentially the same results, although the actual concentrations of magnesium and calcium were four times as great in the first series as in the second. This supports the conclusion that the ratio value of magnesium to calcium, rather than the actual concentration of either, mainly determines the extent of the leaf injury.

The results here noted were secured by means of variations in the ratio of $MgCl_2$ to $CaCl_2$. Since a common anion was employed, the effects may be attributed to the cation ratio of magnesium to calcium. Tottingham, Shive, and Trelease varied the proportions of $Ca(NO_3)_2$ and $MgSO_4$, and so the effects they recorded might have been due to the nitrate-sulphate ratio.

The more general occurrence of injury to the fourth leaf than to the third is strikingly brought out by the graphs for series 2 and 3. Injury was never observed in the coleoptile, nor in the first and second green leaves. The fourth leaf often was affected even though the third escaped. In some cases the fifth or the sixth exhibited injury after all of the others had been free from it. When the plants had grown for a sufficiently long period to allow the development of seven leaves, the number of leaves affected on

each plant was greatest in the cultures supplied with solutions of moderate toxicity. The fourth, fifth, sixth, and seventh leaves of such cultures often showed slight injury, whereas the corresponding leaves did not emerge at all in the cultures that had the most toxic solutions.

The curves of figure 1 show that equivalent magnesium-calcium ratios produced considerably less injury in series 3 than in series 1 and 2. This observation supports the indications recorded in the papers of Tottingham and Shive that the simple ratio of magnesium to calcium does not completely determine the quantitative expression of magnesium injury. The solutions of series 3 have much higher absolute concentrations of calcium and magnesium than those of the other two series. Thus in the solution of series 3 for which the magnesium-calcium ratio is 16.39, the actual concentration of calcium is 5.36 times the corresponding concentration in series 1 and it is 21.44 times that in series 2; the concentration of magnesium in the solution of series 3 is, of course, correspondingly high. With equivalent ratio values, magnesium injury was less pronounced in these cases when the actual contents of calcium and magnesium were very high than when they were relatively low. It is to be noted that the severity of magnesium injury was essentially the same in series 1 and series 2, although the actual concentrations of calcium and magnesium were four times as high in the former series as in the latter. But with the still higher calcium and magnesium contents of series 3, the injury was less severe. These cases suggest that when the actual concentration of magnesium was greatly increased, magnesium toxicity could be checked without increasing the calcium in the same proportion.

Antagonistic salt action illustrated by magnesium injury. The direct relation between the severity of magnesium injury and the value of the ratio of magnesium to calcium in the culture solution appears to afford a clear case of antagonistic salt action. The effect of calcium in diminishing the magnesium injury of wheat seems to be similar to many cases of salt antagonism reported in the literature (Loew, 1892, 1903; Loew and May, 1901; Loeb, 1906; Osterhout, 1922; McCool, 1913; Hansteen, 1910; Trelease and Trelease, 1925, 1926; Eisenmenger, 1928; Barton and Trelease, 1927). Leaf development may thus be used as a criterion of toxicity and antagonism in the same way that growth rate, duration of life, electrical conductivity of tissues, and rate of absorption or excretion of electrolytes have been employed in many studies.

Magnesium injury of the foliage does not occur when the concentrations of magnesium and calcium are properly balanced in the culture solution. The absorption of magnesium is probably retarded when a sufficient proportion of calcium is present in the solution. And we may suppose that

the magnesium which is absorbed from the balanced solution produces no harmful effects, because it is accompanied by a suitable quantity of calcium.

Apparent specificity of magnesium injury. The characteristic deformation of the foliage which has been described as magnesium injury appears to be a specific effect of magnesium toxicity, associated with culture solutions having high magnesium-calcium ratio values. No other solution condition capable of producing identical symptoms was discovered in the extensive studies of Tottingham and Shive, and none seems to have been reported by other investigators. It is true that symptoms similar in some respects to those of magnesium injury have been described by Gericke (1922) as occurring in wheat plants supplied with a simple solution of potassium sulphate, potassium nitrate, or potassium dihydrogen phosphate. The relation of these symptoms to those of magnesium injury is not entirely clear, however. A repetition of Gericke's tests confirmed his observation that the young third leaf emerged from the sheath in an abnormally tightly rolled condition and that abscission of the leaves often occurred. Thus the initial symptoms were undoubtedly similar to those of magnesium injury. But the plants in simple solutions of the potassium salts died after from two to three weeks, and none of them developed the spiral deformity that is the most characteristic sign of magnesium injury.

A striking resemblance to magnesium injury of wheat has been shown by the inflorescences of wheat and rye attacked by the fungus *Dilophospora alopecuri* (Schaffnit and Wieben, 1928). The most pronounced deformities of spikes, leaves, and leaf sheaths of wheat occurred when the plants were attacked simultaneously by *Dilophospora alopecuri* and *Tylenchus tritici*. The spikes were prevented from emerging properly from the leaf sheaths, and the continued growth of the culm produced spiral malformations resembling those of magnesium injury. But the spiral deformation took place only in the inflorescences—not in the young leaves of the seedling plant.

Although symptoms of young seedlings identical with those of magnesium injury have not been described thus far as resulting from toxic agents other than magnesium, we should expect that other chemical elements or compounds would be capable of producing similar abnormalities in the growth of wheat seedlings. The response seems to involve a rather simple type of obstruction to growth, and there is little reason to suppose that magnesium is the only agent that can induce it.

Variations in symptoms correlated with differences in the magnesium-calcium ratio. In the present work no attempt was made to keep quantitative records of the different types of leaf injury that occurred in the various

cultures. Tottingham (1914) classified the injured leaves into those exhibiting slight and those exhibiting severe injury. Shive (1915) also divided the injured leaves into two groups—those which were injured throughout their entire length, and those which were normal except for an injured apical region. A similar classification was adopted by Trelease (1920) in recording the injury: when a spiral was formed or when the whole leaf was affected, the injury was considered severe; when no spiral was formed and the whole leaf was not affected, the injury was considered slight. Considerable difficulty is encountered, however, in the use of two classes of this sort. For example, at a certain time the whole leaf may appear to be affected; but subsequent examination may show that the leaf has grown out of the sheath, so that all but the apical portion has developed normally. In the present study, therefore, exact records were kept only of the number of injured leaves in each culture.

Some observations that were made with regard to the distribution of the different types of injury are interesting, however. It was observed that the most conspicuously developed long spirals occurred in the moderately, rather than in the most severely injured cultures. They were common, for example, among the third and fourth leaves of culture 5 (magnesium-calcium ratio, 9.35) of series 1 and 2. On the other hand, very few cases of long spirals were found in the most severely injured plants (e.g., those of culture 8 with a magnesium-calcium ratio of 50.00); in these plants only a thin, tightly rolled, thread-like portion of the leaf emerged from the sheath. Furthermore, the well developed elongated spirals were of more frequent occurrence among fourth leaves than among third leaves. Slightly injured leaves, in which the apical portion remained rolled while the lower part emerged and developed normally, were of course produced in the greatest numbers in the less toxic solutions, with relatively low magnesium-calcium ratios.

Magnesium toxicity and calcium deficiency

The question may be raised as to whether the growth derangements that have been termed magnesium injury are to be attributed to magnesium toxicity or to calcium deficiency. Sufficient information, however, is hardly available as yet for a definite answer.

It has been clearly shown that injury to the foliage does not occur when the concentration of magnesium is sufficiently low in relation to that of calcium. As the magnesium-calcium ratio value is increased, the injury becomes more and more severe. This relationship in itself suggests magnesium toxicity as the controlling condition. It may be supposed that magnesium is absorbed rapidly enough to be injurious when supplied in solutions that

have a relatively low calcium content; or it may be considered that magnesium exerts its toxic effects when entering the plant unaccompanied by a sufficiently high proportion of calcium. Many studies have shown that simple solutions of magnesium salts are highly toxic to roots, but that this toxicity disappears if a sufficient proportion of calcium is added to the culture solution (see, for example, Trelease and Trelease, 1926). On the other hand, the effects of calcium starvation are relatively slow in making their appearance; the roots of young wheat seedlings make rapid and apparently normal growth for a number of days in distilled water, which of course is devoid of calcium. Sufficient calcium to supply the needs of the plant is probably furnished by the material stored in the seed. These facts seem to support the idea that the abnormality of leaf growth is dependent upon magnesium toxicity.

If the foliage symptoms are to be regarded as due to calcium starvation, we must suppose that sufficient calcium for normal leaf development cannot be absorbed when there is an excess of magnesium in the solution surrounding the roots, or that the calcium, even though absorbed, is unavailable for proper utilization by the plant when accompanied by an excess of magnesium.

The cultures of series 4 seem to throw some light on this question. They appear to furnish evidence that the injury is due to magnesium toxicity rather than to calcium starvation. It will be noted from table 4 that the first solution had a magnesium-calcium ratio of 16.39, and that this solution produced typical injury in 85 per cent of the third leaves. The other solutions contained strontium chloride in increasing concentrations. It is apparent from these data that the addition of strontium chloride had a definite inhibiting effect on the injury to the third leaf. When the fourth leaf is considered, however, no inhibition of magnesium injury is evident. Strontium exerted its own toxic effect. Concentrations of strontium high enough to check magnesium injury of the third leaf were so toxic to the plant that they prevented the fourth leaf from emerging from the sheath.

The observations on the third leaf, however, indicate that strontium was able to delay the appearance of the characteristic symptoms of magnesium injury. The partial inhibition of magnesium injury by strontium points to the conclusion that this injury really is due to magnesium toxicity rather than to calcium starvation.

Studies of the influence of variations in the total concentration of the culture solution afford additional evidence against the idea that calcium deficiency produces the growth derangements that have been termed magnesium injury. It has been found that reducing the calcium concentration

in the external solution by diluting the culture solution does not increase the severity of the injury. On the contrary, it appears to decrease it. Thus Shive and Tottingham reported that no coiling of the leaves occurred in their suboptimal solutions corresponding in osmotic value to 0.1 and 0.05 atmosphere.

Magnesium injury as influenced by substances other than magnesium and calcium in the culture solution

Although the magnesium-calcium ratio seems mainly to determine the occurrence and severity of magnesium injury, there is evidence that this injury may be influenced by other substances present in the solution. Thus Tottingham (1914) observed that serious leaf injury increased rapidly as the partial concentration of KH_2PO_4 increased in his solutions of high total concentration (8.15 atmospheres). He points out that this indicates an inter-relation of all solutes in determining the harmful effects of magnesium. It is suggested that the acidity of KH_2PO_4 may have been influential.

Shive's triangular diagrams representing the distribution of severe and slight leaf injury in his optimal (1.75 atmospheres) and supra-optimal (4.00 atmospheres) solutions also show that the severity of the injury produced by a given magnesium-calcium ratio increases with an increase in the partial concentration of KH_2PO_4. Similarly, in another study it was found that the injury extended to lower values of the magnesium-calcium ratio when KCl was present in the solution in high enough partial concentrations to constitute eight-tenths or nine-tenths of a total osmotic value of 1.60 atmospheres (Trelease, 1920).

The results of these studies indicate that the injurious effect of magnesium is controlled by the complex balance between magnesium and all of the other substances present in the culture solution. Magnesium toxicity may be thought of as mainly determined by the magnesium-calcium ratio, but as also influenced somewhat by the proportions and concentrations of other materials present in the solution.

Relation of magnesium injury to the total concentration of the culture solution

Characteristic magnesium injury, involving a spiral deformation of the foliage, has not been reported as occurring in very dilute solutions, even though these have high magnesium-calcium ratio values. Thus Tottingham (1914) found that only one young third leaf exhibited magnesium injury in his series of cultures with a total osmotic value of 0.05 atmosphere. Although this one case developed into a severe stage, no

further trace of injury became visible in this series of cultures; this single case of injury may have been due to a specific weakness or susceptibility of the particular leaf involved. Pronounced injury, however, appeared in the cultures of the series of higher osmotic value—2.50 and 8.15 atmospheres. Shive (1915) also noted marked injury in his cultures with osmotic values of 1.75 and 4.00 atmospheres, but none in the corresponding cultures supplied with solutions diluted to 0.1 atmosphere.

Severe magnesium injury, involving coiling of the leaves, occurred in all tested concentrations, from 7.0 atmospheres to 0.5 atmosphere, of a solution containing KCl, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ and having a magnesium-calcium ratio value of 13.43 (Trelease, 1920). The severity of the injury as well as the number of leaves affected increased with an increase in the total concentration. Even the lowest concentration that was tested (0.5 atmosphere) was high enough to produce the typical magnesium injury.

Some incomplete series of cultures, grown during the spring of 1931, have shown that injury of the young third and fourth leaves occurred when the solution was diluted to 1/4, 1/16, and 1/64 of an atmosphere; but the most conspicuous and characteristic form of injury, marked by a coiling of the leaves, was not found in the most dilute solutions. In these, the young leaf emerged in a tightly rolled condition, but the development did not proceed to the stage in which a well developed spiral is formed. Cessation of growth before the fourth leaf had completely emerged made it very difficult to obtain an accurate record of magnesium injury. Further work will be necessary before a satisfactory report may be made concerning this phase of the problem.

Relation of magnesium injury to climatic conditions

Remarkably consistent results were obtained in series 1 and 2, although these series were conducted at different seasons of the year. The degree of injury to the third leaf in these tests was nearly the same under very different sets of climatic conditions. An earlier study, however, suggested that the occurrence of the injury might have been influenced by aerial conditions (Trelease, 1920). A solution with a magnesium-calcium ratio of 1.72 and a total concentration of 1.60 atmospheres produced slight magnesium injury when used from January 11 to February 4, of 1916, but produced no injury when used from January 23 to February 24 of the following year. The fact that the aerial conditions were different may possibly explain why the injury was not apparent in the second test. Gericke (1922) states that magnesium injury presents itself in much greater degree under conditions conducive to high transpiration of the plants than to low.

Magnesium injury in other plants

Oats, rye, and barley were found to exhibit symptoms of magnesium injury identical with those described for wheat. This observation was made on cultures of the other cereals grown with the wheat cultures of series 1 and supplied with solution 6. All of the characteristic symptoms of magnesium injury developed in plants of these cultures. A series of corn cultures (*Zea Mays*) conducted in the spring of 1931 showed that this plant also develops signs of injury that resemble those of the small grains. Pronounced leaf distortion occurred in solutions of high magnesium-calcium ratios. Spiral deformation was less common, however, in corn.

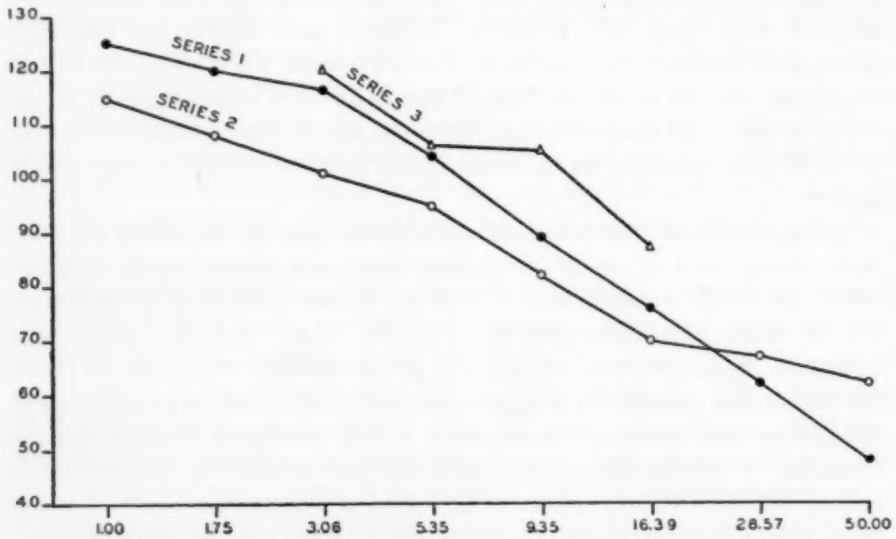

Fig. 2. Dry weights of tops of wheat plants as related to the magnesium-calcium ratios of the culture solutions. Abscissas represent magnesium-calcium ratio values plotted on a logarithmic scale. Ordinates represent dry weights in milligrams.

Graphs for series 1–3 are plotted from the data of tables 1–3.

Tobacco also develops symptoms that resemble in some respects those of the grasses (Garner, McMurtrey, Bowling, and Moss, 1930). Tobacco plants that were grown in soil cultures with a very low supply of calcium in relation to that of magnesium exhibited striking pathological modifications of the upper leaves; the aborted leaves had large indentations in their margins and their tips were lacking. The growing points were affected to such an extent that in most cases the plants were unable to flower.

DRY WEIGHTS OF TOPS IN RELATION TO THE MAGNESIUM-CALCIUM RATIO

The dry weights of tops for series 1–3 are plotted as graphs in figure 2, in which ordinates represent mean weights in milligrams and abscissas

represent magnesium-calcium ratios plotted on a logarithmic scale. The most striking feature of these graphs is the nearly linear decrease in dry weight with an increase in the logarithm of the magnesium-calcium ratio. Thus dry yields of tops are inversely proportional to the logarithm of the magnesium-calcium ratio (between the ratio limits 1.00 and 50.00). In considering the somewhat higher yields for series 1 than for series 2, it should be remembered that these cultures were conducted at different seasons of the year; the higher values of the first series are not necessarily correlated with the fact that actual concentrations of magnesium and calcium were four times as great in this series as in the second. The graph for series 3, while exhibiting some irregularity, lies definitely above those for the other two series. The solutions of this series had much higher actual concentrations of magnesium and calcium than those of either of the other series. It is clear from this comparison that the actual dry weights that may be secured depend not only upon the magnesium-calcium ratio but also upon other characteristics of the solution.

No information regarding optimal magnesium-calcium ratios for growth is available from this study. The optimal ratio would have been 1.00 or lower, for these experimental conditions. Since the aim of the present work was to study magnesium injury, solutions of low magnesium-calcium ratio values were omitted. Other studies (Tottingham, 1914; Shive, 1915; Trelease, 1920) have shown that the dry yield is markedly reduced when solutions of very low magnesium-calcium ratio values are employed. Pathological symptoms of wheat which are quite different from those of magnesium injury may occur under these conditions; the symptoms that have been noted include bleaching of the tissues between the main veins and a general chlorosis of this foliage. The green color of the leaves becomes less intense with decrease in the magnesium-calcium ratio. The largest tops are intermediate in color. Garner, McMurtrey, Bacon, and Moss (1923) have shown that pronounced chlorosis of tobacco, known as sand-drown, is associated with magnesium deficiency. But how closely it is correlated with the magnesium-calcium ratio has not as yet been thoroughly investigated.

An antagonistic effect of strontium toward magnesium seems to be brought out clearly by the dry weights shown in table 4. The addition of successively higher concentrations of strontium is accompanied by a progressive increase in dry yield, until a maximum yield is reached when the solution contains 0.001 M $SrCl_2$. A further increase in the concentration of strontium results in a reduction of yield, due no doubt to the toxicity of the strontium.

SUMMARY

Magnesium injury of wheat becomes visible when the young third leaf is emerging from the sheath. The apical portion of this leaf remains tightly rolled. The most characteristic abnormality is a spiral deformation that is produced in the lower part of the young leaf, enclosed within the sheath. Emergence of the leaf is checked, and continued growth pressure from below crushes a considerable length of the leaf into a close spiral. Later the spirally distorted region may emerge from the sheath, followed by a rolled portion. Abscission of the tightly rolled, needle-like tips of injured leaves frequently occurs. The characteristic leaf deformation, marked by a spiral distortion of the young leaves, appears to be a specific symptom of magnesium toxicity.

Injury was not observed in the coleoptile, nor in the first and second green leaves. It was much more general in the fourth leaf than in the third. In some cases the fifth or the sixth leaf exhibited injury after all of the others had been free from it.

Other derangements accompany the deformation of the foliage. The leaves are darker green than those of normal plants, numerous tillers develop prematurely, growth of the seminal roots is inhibited, and a number of adventitious roots are produced.

The occurrence and severity of magnesium injury are mainly determined by the ratio of magnesium to calcium in the culture solution. This was shown by an increased percentage of injured leaves as the magnesium-calcium ratio increased from 1.00 to 50.00. Magnesium injury thus appears to afford a clear case of antagonistic salt action.

The leaf injury seems to be due to magnesium toxicity, rather than to calcium deficiency. This conclusion is supported by the observation that strontium was able to delay and partially inhibit the characteristic symptoms of magnesium injury.

Magnesium injury, while mainly controlled by the magnesium-calcium ratio, appears to be influenced by the proportions and concentrations of other materials present in the culture solution, as well as by the total concentration of the solution and possibly also by climatic conditions.

Oat, rye, and barley seedlings exhibited symptoms of injury identical with those observed in wheat. Corn also developed signs of injury that resembled those of the small grains.

The dry weights of tops of wheat plants were found to be inversely proportional to the logarithm of the magnesium-calcium ratio, between the limits 1.00 and 50.00 of this ratio.

An antagonistic effect of strontium toward magnesium was brought out clearly in this study. The addition of successively higher concentrations

of strontium was accompanied by a progressive increase in dry yield, until a maximum was reached; further increase in the concentration of strontium resulted in a reduction of yield due to the toxic action of the strontium.

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Explanation of plates

PLATE 5

Fig. 1. Wheat plant exhibiting severe magnesium injury. Tillers have developed prematurely, and young leaves remain rolled. Series 2, culture 5. ×2.75.

Fig. 2. Early stage of magnesium injury, showing emergence of tightly rolled young leaf. Apical portion remains as stiff, wire-like structure. In many cases abscission of this leaf tip occurs. If injury is very severe, growth of the young leaf soon ceases. Injured fourth leaf of series 2, culture 4. ×2.75.

Fig. 3. Emergence of spiral from sheath. Spiral and rolled apical portion assume a drooping position. Fourth leaf of series 3, culture 4. ×2.75.

PLATE 6

Fig. 4. Spiral deformation, showing details of coil. Direction of coiling is reversed several times, with compact, irregular loops separating the more perfectly formed spiral regions. Fourth leaf of series 1, culture 5. ×7.0.

Fig. 5. Spirally distorted portion between tightly rolled apical and basal regions. Fourth leaf of series 3, culture 3. ×6.4.

Fig. 6. Leaf with two spirals. This type is rare. Fourth leaf of series 1, culture 5. ×2.2.

Fig. 7. Apex of fourth leaf emerging for short distance out of opening at the top of the sheath. Emergence of the lower part of the leaf has occurred through the side of the sheath. Series 2, culture 4. ×2.75.

PLATE 7

Fig. 8. Spiral in fourth leaf of series 1, culture 5. ×7.0.

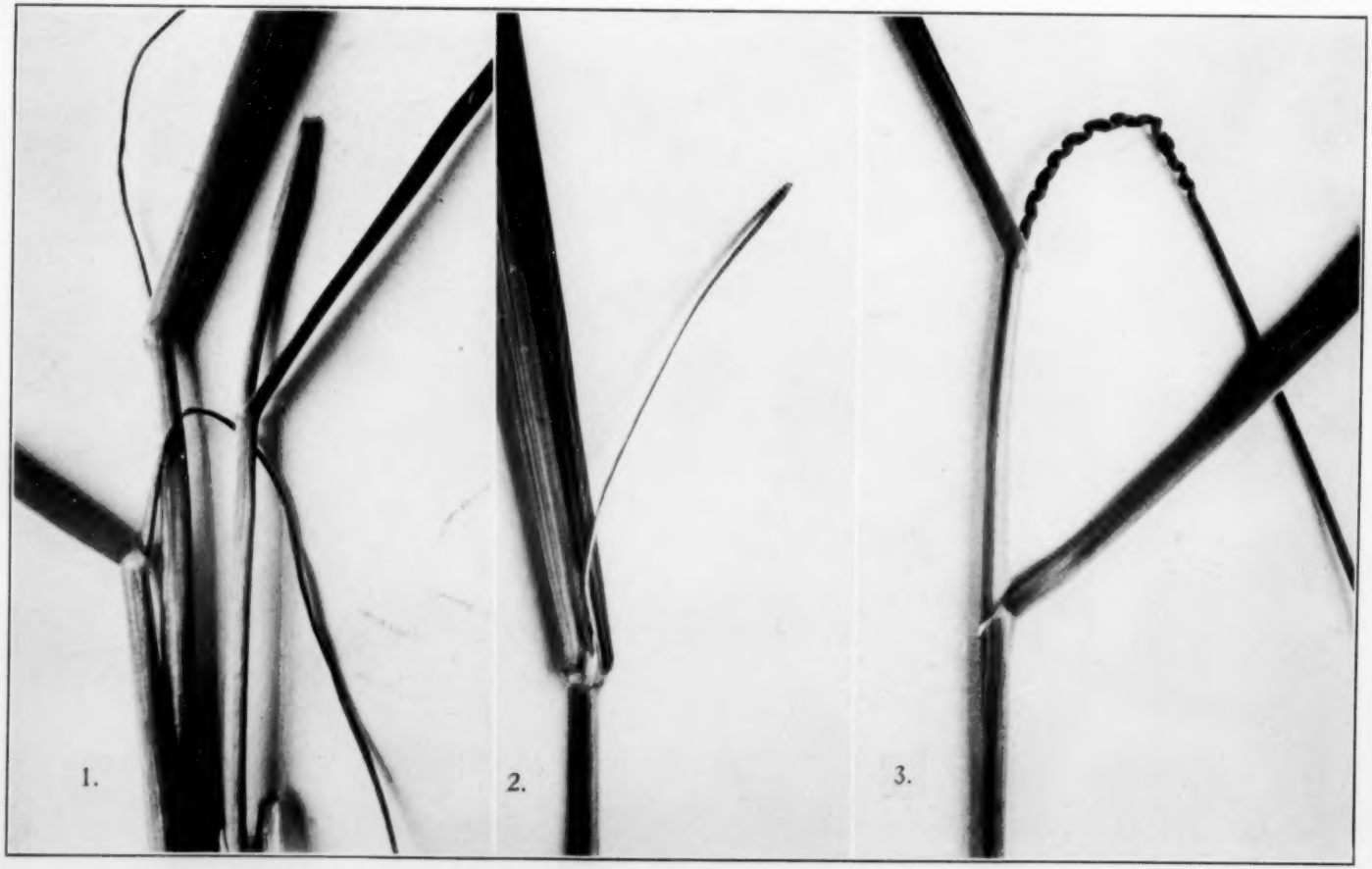
Fig. 9. Leaf in which emergence of tightly rolled apex and spiral has been followed by rapid emergence and normal expansion of lower part. Fourth leaf of series 1, culture 5. ×3.5.

Fig. 10. Slightly injured leaf, showing long, loose spiral, with normally expanded lamina below. Marginal laceration extends to midrib. Fourth leaf of series 1, culture 3. ×2.2.

Fig. 11. Very slightly injured leaf. No spiral has been formed. Apex has remained tightly rolled, but rest of leaf has unrolled normally and has attained the usual length of healthy leaves. Fourth leaf of series 1, culture 4. ×2.2.

PLATE 8

Appearance of cultures of series 2 at time of harvesting. From left to right: Top row, cultures 8, 7, 6, and 5; bottom row, cultures 4, 3, 2, and 1. (Small numbers in photographs represent original culture numbers; to facilitate presentation of results the order has been reversed in this paper.) Decrease in size of plants (in passing from culture 1 to culture 8) is correlated with increase in value of magnesium-calcium ratio.



TRELEASE AND TRELEASE: MAGNESIUM INJURY OF WHEAT

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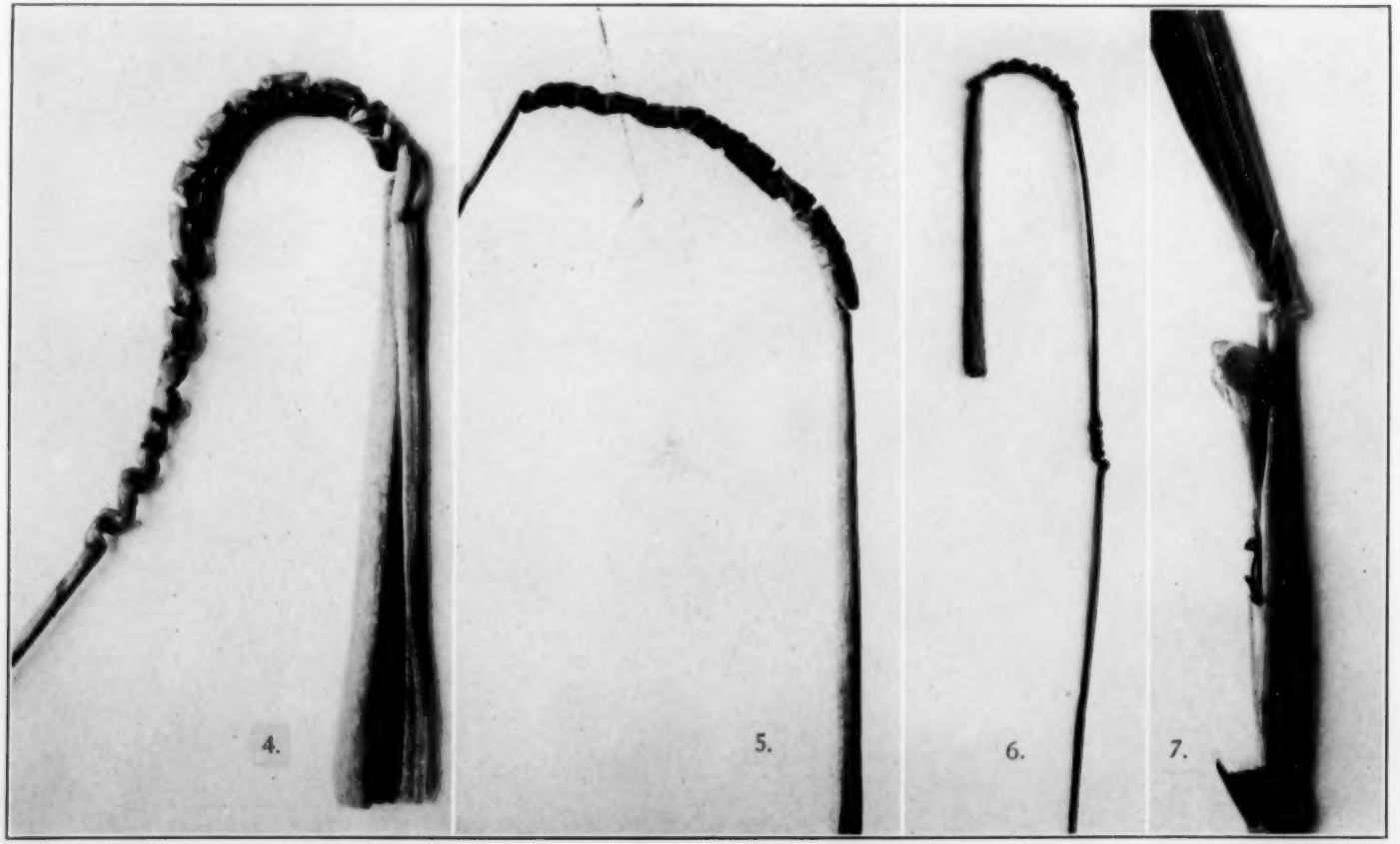

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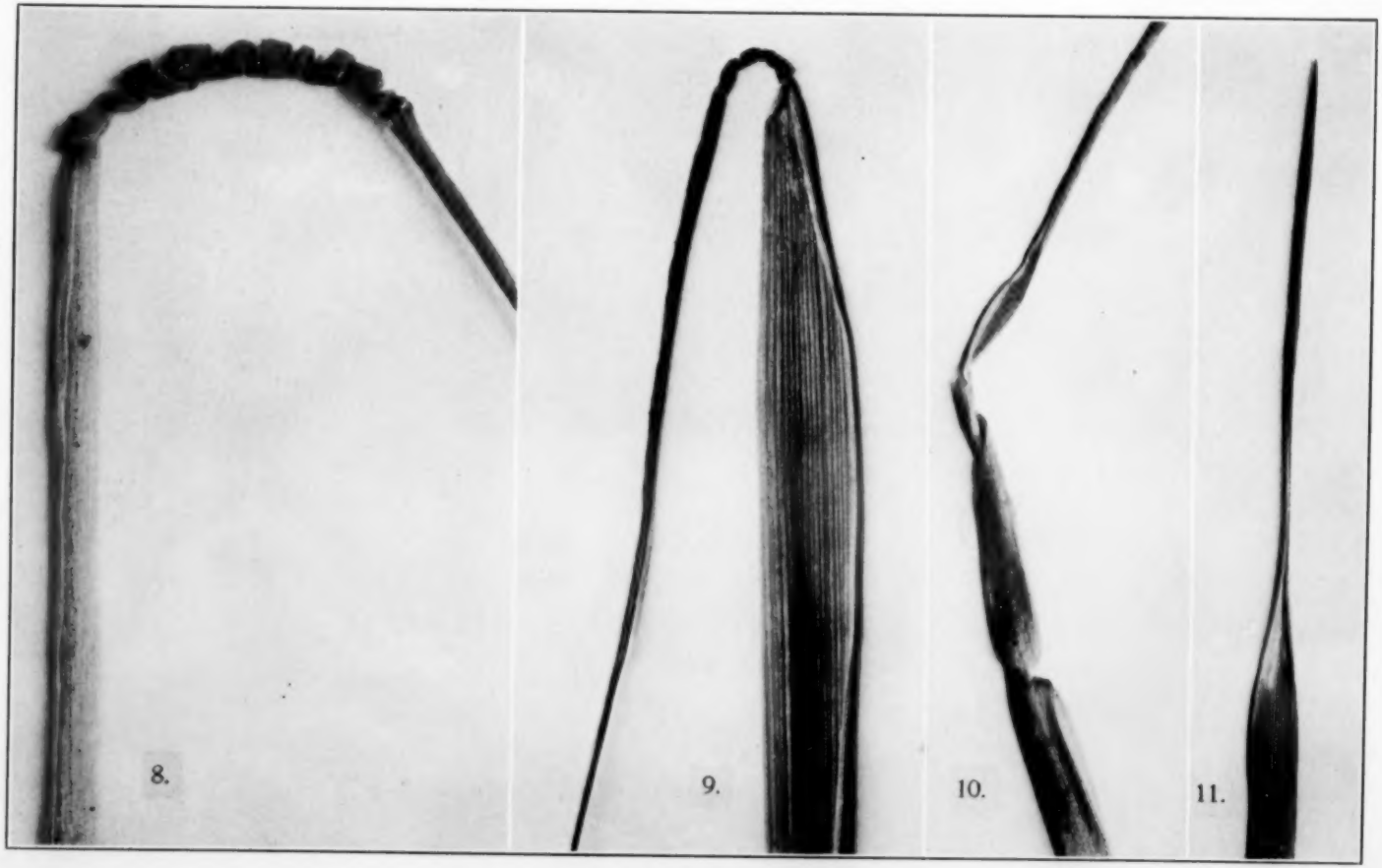

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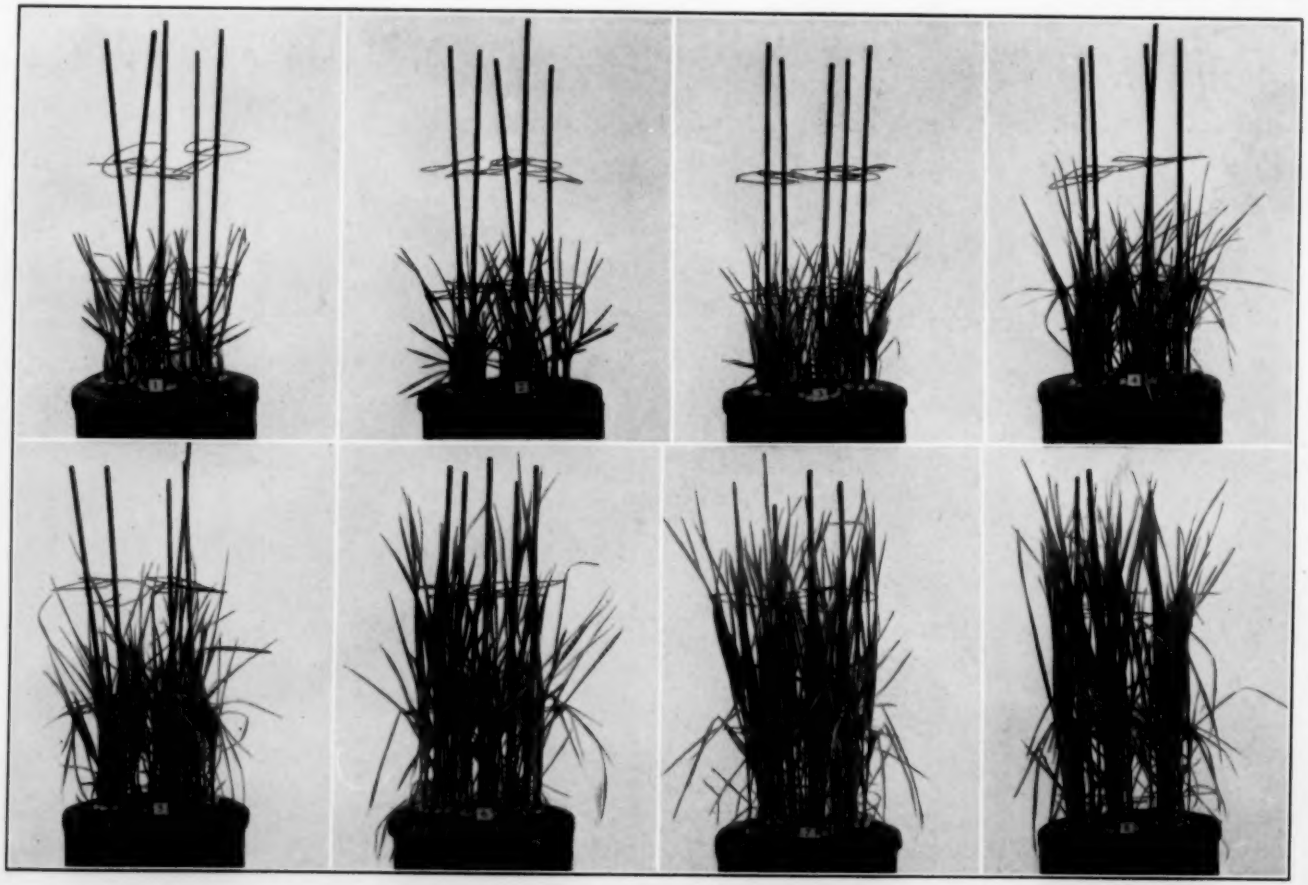

TRELEASE AND TRELEASE: MAGNESIUM INJURY OF WHEAT

Changes in root tips of wheat and corn grown in nutrient solutions deficient in calcium

RONALD BAMFORD

(WITH TWO TEXT FIGURES AND PLATES 9–13)

The rôle of the various mineral nutrients in plant growth has attracted the attention of investigators for over a century. Because of its pronounced effect on plant growth, calcium is one of the most interesting of the essential mineral elements. Many of the earlier workers recognized that the roots of plants grown in solutions deficient in calcium remain dwarfed, develop few lateral branches, and in time become badly discolored. On the other hand, if small quantities of this element are present in the culture solutions, these conditions do not appear or, at least, their appearance is greatly delayed. It is the purpose of this paper to report the results of a periodic examination of the cytological condition of root tips from corn (*Zea mays* L.) and wheat (*Triticum vulgare* Vill.) seedlings which were grown in solutions deficient in calcium. This study was made in an attempt to add to our knowledge concerning the function or physiological rôle of this element in the root cells of these plants.

The literature on the rôle of calcium in plant growth has been reviewed at frequent intervals. Loew (1899, 1901), Reed (1907), Warthiadi (1911), True (1922), Arrhenius (1926), Mevius (1927), and Farr (1927b) may be cited as among those who have presented extensive reviews of the literature.

Salm-Horstmar (1856) seems to have been the first to show the necessity of calcium for plant growth and the fact that it could not be replaced by magnesium. Stohmann (1862) demonstrated the necessity of calcium for the successful cultivation of corn plants, and shortly afterward Wolf (1864) noted that solutions of calcium salts accelerated root growth, in contrast with solutions of magnesium, potassium, ammonium, and sodium salts, which retarded it. Following these earlier investigations there were many speculations as to the function of this element in cellular metabolism. Holzner (1867) considered that it was necessary for the formation of cellulose, but later work by Mangin (1892) and others demonstrated that it was a constituent of the middle lamella of the cell wall. However, in a recent analysis of the cell walls in the meristematic regions of certain plants, by Tupper-Carey and Priestley (1923), calcium appears to be ab-

sent from the middle lamella, although they found it present in this part of the cell walls in the mature parts of the plant.

Boehm (1875), von Raumer and Kellerman (1880), and von Raumer (1883) considered its chief function as that of aiding in the transportation of the starch produced in photosynthesis. Groom (1896) claimed that carbohydrates were moved regardless of the presence of calcium in the tissues, though he considered that calcium compounds facilitated their movements. More recently, however, Maquenne and Demoussey (1922) have reported that calcium is the only element not necessary for the successful germination of pea seeds—a process in which the transportation of seed carbohydrates is essential.

Loew (1892, 1899, 1901) was perhaps the first to stress the essential nature of calcium for normal development in all the higher plants, although his hypothesis has not been accepted by many investigators. He concluded that calcium functions principally in two ways: (1) it combines with the proteins in the formation of nucleo-proteins, which take part in the construction of the nucleus, and (2) it neutralizes and precipitates the harmful acids in the cell sap, chief of which is oxalic acid. His second contention had been advanced previously by Schimper (1890). Warthiadi (1911), after a series of extensive observations on *Tradescantia*, agreed with Loew. Essentially the same explanation has been offered recently by Parker and Truog (1921). They found that high protein content accompanied high calcium content, and they assumed that the calcium neutralized an acid condition supposed to be brought about during the manufacture of these proteins. This theory has been severely criticized on the ground that fungi do not require calcium and still are able to produce large quantities of proteins and organic acids.

Although Boehm (1875) had noted that the toxicity of certain salts could be lessened by small quantities of calcium, Loew seems to have been the first worker to study this phenomenon extensively. He thought that the magnesium displaced calcium in parts of the protoplast when that element was absent from the culture solution; also that calcium took the place of magnesium when that was absent, with the result that phosphoric acid could not be transported. In either case the normal action of the cells was inhibited. Upon this he framed his well-known hypothesis of "the calcium-magnesium ratio," which stated that a specific ratio of calcium to magnesium was necessary for each type of plant, and that maximum development of the plant could only be reached when these elements were supplied in the optimum ratio. This hypothesis is still the subject of controversy, although the predominating evidence, aside from that furnished by Loew and his students, has been against the acceptance of it. McCool

(1913) and Lipman (1916) have presented summaries of the literature on this question. It is interesting to note that Haselhoff (1893), Molisch (1895), and Loew (1901) all believed that the toxicity of magnesium could be diminished by calcium but not by strontium and barium.

Kearney and Cameron (1902) and True and Geis (1903) were apparently among the first plant workers to recognize that the antidoting powers of nutritive elements were not confined to calcium. At this time the conception of antagonism and physiologically balanced solutions had been advanced by Loeb (1900) after his study on marine fishes. Shortly afterward Osterhout (1906, 1912) confirmed and extended Loeb's work by his extensive investigations on many forms of plants, including marine and freshwater algae, liverworts, and seed plants. In contrast with Loew's views that the function of calcium in reducing the toxicity of magnesium is an internal one, they considered that the important action of this element, along with many others, takes place at the surface of the protoplast. Antagonistic substances, present in a balanced solution, act upon the surface of the protoplast in such a way as to mutually retard the entrance of one another into the cell. Loeb (1911) stated that antagonistic salts may have a "tanning" effect on the surface films of cells, whereby these films acquire those physical qualities of durability and comparative permeability, without which the cell cannot exist. Osterhout's views, the results of many experiments, are summed up in his monograph (1922). He offers a detailed hypothesis of the mechanism of antagonistic action and shows that measurements of the electrical conductivity of tissues immersed in simple solutions enable one to predict what substances will antagonize each other and what degree of antagonism may be expected. Further indication of the surface effect of antagonistic ions was furnished by Hansteen (1910) when he grew a seedling in two different culture solutions by dividing the roots, and found that a plentiful supply of calcium from one solution did not reduce the toxic action upon the roots that were immersed in a simple solution of a magnesium salt. Although the principle had been emphasized much earlier by Loeb and Osterhout, McCool (1913) demonstrated clearly that antagonistic action was displayed by non-essential elements, such as strontium, barium, and sodium in the growth of crop plants; but calcium was the most effective of the elements that he tested. A valuable discussion of the literature on the subject of antagonism is offered by Stiles and Jorgensen (1914).

Another line of evidence as to the function of calcium is furnished by the work on the toxic action of distilled water. True (1914) observed that distilled water inhibited root growth even if the water contained no traces of toxic metallic salts. He found that when lupine seedlings were placed in

distilled water the electrolytic conductivity of the water increased. His explanation was that the distilled water brought about the dissociation of the electrolytes from their points of combination in the proteins. The loss of these electrolytes from the roots caused an increase of the conductivity of the water and was ultimately responsible for the death of the cells. He also found that if a calcium salt were added to the water in sufficient quantity to make a solution osmotically equal to tap water, then the leaching of electrolytes did not take place. Merrill (1915a, 1915b) came to similar conclusions concerning the conditions responsible for the increase in electrolytic conductivity, but claimed that the death of the cells was chiefly due to secondary factors that affected the cells in their starved condition. He was able to increase the exosmosis of electrolytes by treating the roots with various anesthetics for very short periods of time. Hibbard (1915) also found that the electrolytic conductivity of distilled water was increased by excretion from roots. He suggested that the death of the cells might be due to the toxicity of substances excreted by the roots into the culture solution. He used the extensive observations of the United States Department of Agriculture, initiated by Schreiner and Reed (1907), on toxic soils, to support his suggestion. Scarth (1924) offered another explanation of the toxicity of very pure distilled water. He considers the hydrogen-ion concentration of the water to be responsible, explaining that the acidity of the water is increased by dissolved carbon dioxide. It is interesting to note that calcium antagonizes this toxic action.

True and Bartlett (1912, 1915a, 1915b, 1916), after subsequent experiments, elaborated on True's original idea that calcium prevented the excretion of electrolytes from the roots of lupines. He also found that other cations, at certain concentrations, aided in preventing this loss of electrolytes. However, calcium was the most effective of these cations, regardless of whether in a one-, two-, or three-salt solution. In later papers (1921, 1922) True emphasized his conclusion that calcium is the most active agent in preventing leaching from the roots of lupines, and he suggested also that calcium plays an important part in cell wall formation and in the differentiation of tissues. He considered that the rôle of calcium in cell wall construction may even be linked with its action in preventing the toxic action of other elements upon the cell. Thus, he explained, as a result of experiments by Dr. Eckerson in his laboratory, that when calcium is absent from the culture solution, magnesium or potassium replaces the calcium of the middle lamella and forms magnesium or potassium pectate, thereby subjecting the cell to the toxic action of magnesium or potassium.

The evidence presented by various workers is conflicting, however, and no general hypothesis seems to explain all of the experimental data. For

example, Tuewa (1926) reported that when the hydrogen-ion concentration of the nutrient solution was kept at a pH value of seven or above, exosmosis did not occur. This seems to be in direct opposition to the observation of Prianischnikow (1923) that the injurious action of such acids as nitric or hydrochloric, in weak concentrations, could be reduced by the addition of a calcium salt, even though the hydrogen-ion concentration remained the same.

Brooks (1916), investigating the exosmosis in the dandelion peduncle, found, after testing the chlorides of calcium, sodium, and cerium, that calcium was the only cation which decreased the rate over very short periods of time. Osterhout (1923) employed the electrical resistance of the protoplasm to study the changes occurring in the protoplasm while active exosmosis was going on and concluded that active exosmosis indicated injury to the cell. Stiles and Jorgensen (1915, 1917) found that the more toxic the ions and the higher the concentration of those ions, the higher the rate of exosmosis.

True and Merrill both suggested that their methods of measurement by electrical conductivity did not take into account the non-electrolytic substances which might be excreted by the tissues and cells of the root. Very few observations have been made to determine the exact nature of all the excreted products, although many writers have assumed that some of the materials may be organic. Knop (1864) noted that seeds excreted both inorganic and organic substances into pure water. Similar observations were made by Hansteen (1894) and Puriewitsch (1897). Wächter (1905) obtained the exosmosis of sugar from onion scales after immersion in distilled water. He was able to reduce this if small amounts of potassium or sodium chloride were added to the water. On the other hand, ether treatment produced a greatly increased excretion which he attributes to the death of the cells. Lepeschkin (1906) was able to obtain similar results with *Pilobolus* spores. Hansteen Cranner (1922) made extensive analyses of the excreted substances from various types of cells and found that phosphatids made up the greater part of the organic substances excreted. This discovery led Hansteen Cranner to suggest that calcium compounds, since they seemed effective in reducing the quantity of the material excreted into the culture solutions, were active in coagulating the lipoid constituents of the protoplasm and thus were able to reduce the entrance of toxic agents. Stiles (1927) found that both organic and inorganic substances were discharged into distilled water from cubes of fleshy roots and tubers. He was able to reduce this excretion by aerating the medium and thought that these products may come from the injured cells on the cut surfaces. Recent investigations by Iljin (1928) on the exosmosis of carbohydrates

and potassium have served to emphasize the importance of calcium in controlling this process. He found that this element, whether in a one-, two-, or three-salt solution, was the most effective in reducing exosmosis.

Osterhout (1923) suggested that the greatest exosmosis, brought about by a toxic agent, probably occurs during the death of the cell. If this is so, we should expect to find organic as well as inorganic constituents among the excretion products. The whole question of the excretion of materials from cells has been considered in the literature from the standpoint that greater permeability of the membrane, regardless of what conception we may have of the structure of this membrane, causes increased exosmosis. Szücs (1912), however, doubts whether we should in general regard exosmosis as due to protoplasmic permeability, because in many cases the phenomena observed are not concerned with vital processes, but are the results of the death of the cells. Stiles (1924), after an excellent discussion of the literature on the subject, suggests that in some cases no change may take place in the membrane, but that complex and indiffusible substances may break down into simpler and diffusible ones. An interesting point to note in this connection is that neither Hansteen Cranner (1922) nor Iljin (1928) was able to get a protein test in their analyses of the substances excreted into culture solutions.

Not all the studies on exosmosis indicate that this process is such as to cause the death of the cells of the tissue involved. Merrill (1915a) reported that he was able to get seedlings to resume normal growth even after extended exposures to distilled water for twenty days, and Steward (1928), after protracted leaching experiments of forty-five days duration, found that slices of beet could form wound tissue, thus indicating that the cells, had remained in a semi-permeable condition throughout the experiment.

Direct observations on the effects of calcium on cell structure were first reported by Molisch (1895) on *Spirogyra*. Reed (1907) confirmed the findings of Molisch and observed that in the absence of calcium *Spirogyra* cells underwent nuclear division but cytokinesis failed and thus new cell walls were not formed. Similar observations were made on the root cells of *Zea mays*. He explains that the absence of new cell walls after mitosis in cells lacking calcium indicates that the cells were unable to form the fundamental 'middle layer' of the cell wall and, in consequence, no cell was formed. Loew (1899), in discussing Molisch's observations, offered a similar explanation. Hansteen Cranner (1914) reported that individual cells or rows of cells become separated from the surface of the root in the elongation zone, due to the dissolution of the cell wall in the absence of calcium. Later observations (1922), already mentioned, disclose that he changed his conception of the function of calcium. It should be pointed out

that Hansteen Cranner (1914, 1919, 1922, 1926) has always stressed the cell wall as an important factor in determining cell permeability. This conforms with his conception of the cell wall as being made up partly of protoplasmic infiltrations that are continuous with the cell protoplast.

Sorokin and Sommer (1929), in an intensive study of the root tip cells of *Pisum sativum* which were grown in culture solutions deficient in calcium, found that the death of the plants was associated with the degeneration of these meristematic cells. The degeneration was marked by the appearance of large vacuoles and the consequent decrease in the amount of cytoplasm. These changes disturbed mitotic division, with the result that pseudoamitotic and even typical amitotic division occurred. The abnormal divisions sometimes resulted in the formation of binucleated cells. The authors suggested that the deranged mitoses might be explained by assuming either that calcium is a necessary chemical constituent of the protoplast or that it is essential for the maintenance of the normal colloidal system of the cell. Day (1929), after an examination of the mature roots of the same species, reported that there were no signficant changes in the mature structure of the plants grown in solutions deficient in calcium.

Another conspicuous effect of calcium seems to be that of stimulating the growth of root hairs. Magowan (1908), Hansteen (1910), Coupin (1917) Kisser (1925), and Trelease and Trelease (1926a) all observed that in a single-salt solution containing calcium, dense growths of root hairs were formed, while in distilled water and in simple solutions lacking calcium few or no root hairs developed. Farr (1927a, 1927b, 1927c, 1928a, 1928b, 1928c) found that all calcium salts which he tested accelerated the growth of root hairs of Georgia collards, but that the anions of the salts were also important. All these results were produced with relatively weak concentrations of the salts. Addoms (1927) observed that if strong concentrations of calcium salts were used, the root hairs of wheat underwent radical protoplasmic changes within a few minutes. Toxic action was indicated by coagulation and flocculation of the protoplasm. Along similar lines Brink (1925) found that pollen tube growth was stimulated by the addition of suitable amounts of calcium salts to the culture media.

The relation of calcium salts to transpiration has received some attention. Burgerstein (1920, p. 97–105) has summarized much of the literature. While small amounts of various salts in mixed culture solutions may increase the rate of transpiration over that of plants in distilled water, this rate decreases when the total concentration becomes high. According to Reed (1910), Hansteen Cranner (1914), Chancerel (1914), and Kisser (1927) calcium salts in dilute simple solutions caused an increase in the transpiration rate. Meyer (1931), however, found that calcium salts, in the

concentrations he employed, retarded the transpiration of cotton plants. Iljin (1922) noted that calcium salts check the opening of the guard cells while potassium and sodium salts seem to stimulate this action. This suggests that calcium might be expected to lower the transpiring power of the plants.

The penetrability of the calcium ion into plant cells has received considerable attention, and the generally accepted view is that it enters plants much more slowly than many other ions—more slowly than potassium, sodium, nitrate, chloride, etc. Fitting (1915), Tröndle (1918), and Scarth (1925) have come to this conclusion. Osterhout (1910), however, has shown that it penetrates rapidly enough to permit a ready demonstration of its entrance. Stiles and Kidd (1919) have shown that it enters more rapidly at first than later, when an equilibrium tends to become established.

METHODS AND EXPERIMENTS

Wheat cultures. The culture methods employed in this study were essentially the same as those used by others working in this laboratory (Trelease and Trelease, 1925, 1926a, 1926b, 1928; Barton and Trelease, 1927; Eisenmenger, 1928; Cotton, 1930). A predetermined quantity of Marquis spring wheat (supplied by the University of Saskatchewan, crop of 1930) was soaked in distilled water for two hours and, after a thorough washing, transferred to culture dishes and germinated on wet filter paper until the primary roots were approximately six millimeters long. At this time selected seedlings were placed on paraffined bobbinet which was stretched over each of a number of enamelled iron pans filled with distilled water. Pyrex glass dishes were substituted for the enamelled pans after the first experiment. Each of these dishes was placed in a slightly larger dish, and the space between was filled with distilled water until the level of the water reached the top of the smaller dish containing the seedlings. During the first two days of growth a moist chamber was provided for the seedlings by placing a Pyrex glass cake dish on top of the smaller seedling dish. Two days after removing this glass cover, selected seedlings (the primary root measuring between 4.5 and 6 cm. and the tops between 6 and 7 cm.) were transferred to the culture solutions.

The culture vessels were cylindrical, glazed earthenware jars, obtained from the General Ceramics Company, having a capacity of about 7500 cc. Each jar was nearly filled with culture solution and then covered with a heavily paraffined Portland cement disk having five circular openings, in which were placed the paraffined cork stoppers that supported the plants. Each stopper bore either five or eight seedlings per culture jar. The cultures were kept on rotating tables (Shive, 1915) in the new roof green-

house of Columbia University, and they were therefore subjected to the same fluctuations in environmental conditions throughout the course of the experiment. Artificial light, controlled by a synchronous-motor time switch, was used to supplement daylight from 4 to 10 p.m., except during the first three weeks of the first experiment, which was started before the installation of the lights. When the wheat plants attained sufficient height, they were supported by means of wire loops that were attached to dowels, the lower ends of which were inserted into cork stoppers.

Corn cultures. A similar method was employed with the corn. Golden Eureka Dent corn (*Zea mays* L.) was soaked overnight, and after a thorough washing with distilled water it was germinated in the way described for wheat. When the roots were from one to two centimeters long, the seedlings were placed on paraffined wire netting which covered Pyrex dishes filled with distilled water. They remained there until the roots had attained a length of approximately 4.5 centimeters, and were then put in the culture solutions. One seedling was placed in each paraffined cork stopper, so that there were five plants per culture jar.

Nutrient solutions. In experiment 1, wheat (25 plants per culture) was grown in solutions of three different total concentrations, corresponding to two, one-half, and one-eighth atmospheres of osmotic pressure. These are designated series A, B, and C, respectively. The salts and their proportions are listed below.

	Series A	*Series B*	*Series C*
KH_2PO_4	.005 m	.00125 m	.0003125 m
K_2SO_4	.010 m	.00250 m	.0006250 m
NH_4NO_3	.005 m	.00125 m	.0003125 m
$CaCl_2$ and $MgCl_2$	.020 m	.00500 m	.0012500 m
Total	.040 m	.01000 m	.0025000 m

In each series the ratio of calcium to magnesium was varied from 100/0 to 0/100, as indicated in table 1, by means of eleven different solutions. A total of thirty-three different solutions was thus employed in experiment 1.

Observations and measurements of growth of the roots were recorded, and material was fixed for cytological study at the end of the first four days, at the end of seven days, and then weekly during the remainder of the time.

Experiment 2 was performed by Dr. Sam F. Trelease and through his kindness the writer was enabled to obtain material for cytological study. Corn plants were grown in solutions corresponding to approximately one-half an atmosphere of osmotic pressure. The salts used and their propor-

tions are listed below, while the ratios of calcium to magnesium are presented in table 1. Each of the nine different solutions was duplicated, making eighteen culture jars of plants.

KH_2PO_4	.002 m
NH_4NO_3	.002 m
K_2SO_4	.002 m
$CaCl_2$ and $MgCl_2$	.0025 to .005 m
Total	.0085 to .011 m

Material from this experiment was fixed at the end of twenty-five days.

Experiment 3 was undertaken to supplement the data secured from experiment 1. Wheat seedlings (40 per culture jar) were grown in a solution of the same composition and total concentration as in series B, experiment 1. The calcium and magnesium ratios are listed in table 1. Root tips from four plants were taken for fixation every two days throughout the duration of the experiment, which was two weeks.

TABLE 1

Ratio of calcium to magnesium

CULTURE NO.	EXP. 1.	EXP. 2.	EXP. 3.
1	100/0	63.6/36.4	2/98
2	98/2	50/50	1/99
3	95/5	36.4/63.6	.5/99.5
4	90/10	24.7/75.3	0/100
5	80/20	15.8/84.2	0/100*
6	50/50	9.8/90.2	80/20
7	20/80	5.7/94.3	—
8	10/90	3.5/96.5	—
9	5/95	2/98	—
10	2/98	—	—
11	0/100	—	—

* H_3BO_3, in a concentration of .000045 m, was added to this culture.

General cultural methods. To every culture $FeSO_4$ was added as a source of iron in a concentration of .00001 m. All culture solutions were changed regularly so as to give each wheat plant 60 cc. per day and each corn plant 100 cc. Temperature records and atmometer readings were kept for each of the experiments. These data are presented in table 2.

The hydrogen-ion concentration of the solutions in experiment 1 was taken after the renewal of the culture solutions and then again before the next renewal but no changes were recorded.

Cytological methods. The plant materials were fixed in Flemming's medium fluid and then infiltrated and imbedded by the usual xylol-par-

affin method. Sections were cut 10μ thick and stained with Flemming's triple and Haidenhain's iron-alum haematoxylin stains. The triple stain proved the more favorable for the material concerned.

TABLE 2

Experimental records

EXP.	DATE	TEMP. AVE.	TEMP. RANGE	AVE. DAILY EVAPORATION FROM ATMOMETER
Exp. 1	Oct. 27, 1930 to Dec. 1, 1930	19°C.	14°–25°C.	12.0 cc.
Exp. 2	Jan. 18, 1931 to Feb. 17, 1931	21°C.	18°–25°C.	21.1 cc.
Exp. 3	Feb. 22, 1931 to Mar. 9, 1931	22°C.	15°–27°C.	22.0 cc.

RESULTS

Gross effects

Throughout the experiments special attention was paid to the roots of the plants which were grown in the solutions deficient in calcium—those from which calcium was absent and those in which the content of calcium was low in proportion to that of magnesium. Only brief reference to the tops will be made in this paper, since the investigation was primarily concerned with the roots.

TABLE 3

Average length and dry weight of roots in experiment 1

CULTURE NO.	AVE. ROOT LENGTH IN CM.			AVE. DRY WEIGHT OF ROOTS IN MGM.		
	Series A	Series B	Series C	Series A	Series B	Series C
1	20.4	32.3	22.2	15.0	18.6	18.2
2	21.3	20.4	18.2	19.8	15.4	16.2
3	19.4	19.6	17.2	17.7	14.8	15.3
4	21.5	22.2	17.7	19.4	15.8	15.9
5	20.7	21.6	17.5	21.3	20.0	14.1
6	19.9	21.4	18.1	23.2	19.7	18.7
7	20.9	26.3	18.7	22.0	18.5	19.7
8	22.3	22.9	19.1	24.0	19.9	16.9
9	19.3	24.8	19.7	18.0	18.7	16.5
10	17.5	24.7	16.7	17.1	20.4	15.9
11	5.5	5.7	5.7	4.0	5.3	7.0

Wheat. The root lengths and dry weights of roots of the wheat plants of experiment 1 are presented in table 3, and they are shown graphically in figures 1 and 2. The most obvious feature of both of these figures is the steep slope at the extreme right end of each curve. This indicates a great increase in root growth when the proportion of calcium in the culture solution is increased from 0 to 2 or 5 per cent of the total concentration of calcium and magnesium. Thus we notice that when calcium is absent, the

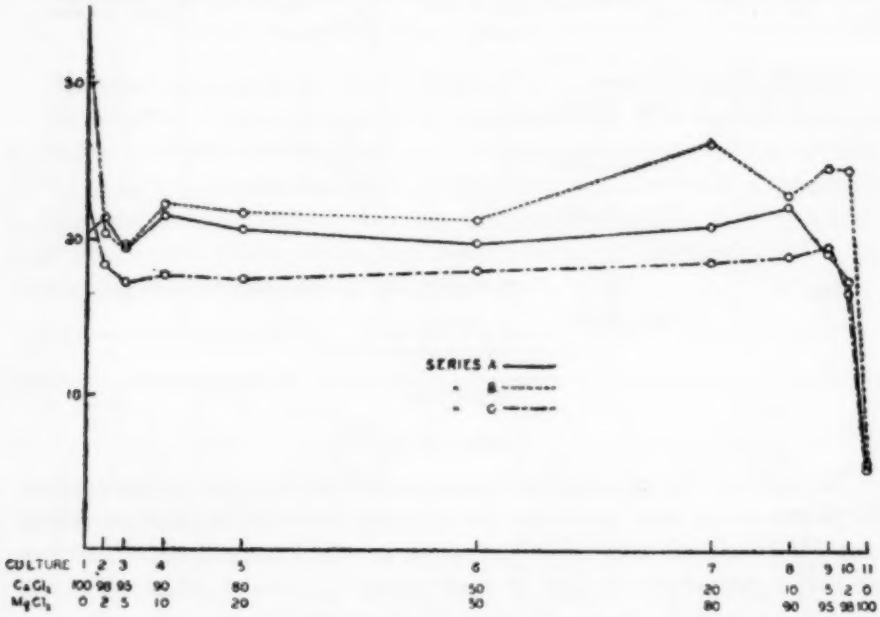

Fig. 1. Average length of primary wheat roots from fifteen plants grown in culture solutions of three different total concentrations.

The ordinates represent growth in centimeters and the abscissas the ratios of calcium to magnesium in each solution of the three different total concentrations.

values for root length and dry weight are very low; but when calcium and magnesium are in a ratio of 5/95, the root lengths and dry weights are practically as great as those obtained with much higher proportions of calcium to magnesium. In fact, the root growth is nearly the same throughout the wide range of proportions of calcium to magnesium lying between 5/95 and 98/2. It is interesting to note that a marked rise in the curves for root length and dry weight occurs when magnesium was absent from the solutions of series B and C (medium and low total concentrations of salts).

Although the experiment was carried on for five weeks, the seminal roots attained their maximum length in approximately two weeks; but the

lateral roots of most of the plants continued to develop throughout the entire period. Rough measurements taken periodically, when fixations were made, showed this to be true.

Frequent observations showed that the roots of the plants in solution 11 (Ca/Mg = 0/100) of each series remained the same as at the beginning as far as length was concerned. Between the ratio values for solution 11 (0/100) and that for solution 9 (5/95) there seems to be a definite critical

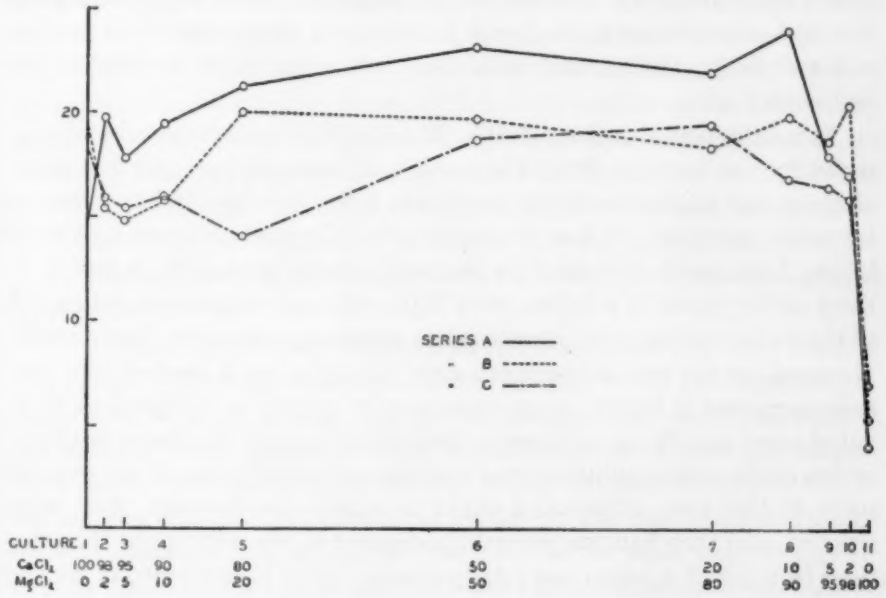

Fig. 2. Average dry weight of wheat roots from fifteen plants grown in culture solutions of three different total concentrations.

The ordinates represent the dry weights in milligrams and the abscissas the ratios of calcium to magnesium in each solution of the three total concentrations.

range of ratio values, within which the growth in length of roots is markedly influenced by the ratio of calcium to magnesium.

A few days after the experiment began, the roots of the plants grown in solution 11 of each series, besides having made no measureable growth, were slightly discolored, crooked, and devoid of lateral branches. Also, the original five seminal or primary roots of each plant had been supplemented by a number of new adventitious roots. At the end of a week the roots were badly discolored, very brittle, crooked, and still devoid of lateral roots. The new adventitious roots soon developed the same symptoms of abnormality that were exhibited by the older roots. Each root tip at this time

had become covered with a hyaline droplet of liquid of a viscid nature; the droplets adhered so closely that they could not be removed by shaking the plants. Microscopic examination showed the droplets to be composed of homogeneous gelatinous material, but it indicated nothing regarding their origin—whether due to gelatinization of cell walls, to exudation of materials from the root cells, or to fungus or bacterial action upon the roots. Soon after the droplets were first noticed, the culture solutions began to exhibit a slight turbidity. Throughout the remainder of the experiment there was no noticeable outward change in the roots except that they became soft and flabby, in contrast with their brittle nature at the end of the first week.

In solution 10 of each series (Ca/Mg = 2/98) the plants developed normally for the first few days. They produced many lateral root branches, although not so abundantly as in cultures 1–9, which had higher and more favorable ratios of calcium to magnesium. The seminal roots and their lateral branches in this solution increased almost as rapidly in length as those of the plants in solutions with higher calcium-magnesium ratios. All of the roots retained their normal white appearance for more than a week. However, at the end of about ten days the tip of each seminal root had been surpassed in length by an abundance of lateral roots that had elongated more rapidly than the main root; this indicated clearly an injurious action of the culture solution upon the root system. The tips of the seminal roots at this time exhibited a slight brownish discoloration, they were crooked, and they had the general appearance of the root tips in the solutions from which calcium was totally absent. Later in the period of the experiment these pathological symptoms became more pronounced, and the tips of some of the older lateral roots developed symptoms of injury that were similar to those first apparent in the apices of the main roots.

The roots of plants grown in solution 9 (Ca/Mg = 5/95) of each total concentration were very similar in their development to those in solution 10, except that the characteristic forms of injury did not become evident till after four weeks of growth in the solution. The growth in length of the seminal roots in this solution was practically the same as that of the roots in solutions having much higher proportions of calcium to magnesium.

The root systems of cultures 1–8 (Ca/Mg ratio varying from 100/0 to 10/90) made healthy and rapid development and exhibited no significant pathological symptoms. They were all essentially alike in rate of growth and in external appearance. Even the roots in culture 1, supplied with a solution containing no magnesium, were not visibly different from those of the remainder of the good cultures. In fact, with the medium and low total concentration of salts (1/2 and 1/8 atm.) greater root growth occurred in

solution 1 than in the solutions containing the lower concentrations of calcium; this surprising result seems to deserve further investigation. No clearly defined optimum solution for root growth is apparent among those tested in this study. Growth is so nearly the same throughout the wide range of proportions of calcium to magnesium, from 100/0 to 5/95, that the average of a very large number of plants would need to be employed in order to determine accurately the optimum solution for root growth. In a general way, the graphs of figures 1 and 2 indicate that 1/8 atm. (series C) is least favorable among the three total concentrations tested; while with most Ca/Mg ratios 1/2 atm. (series B) is most favorable by the criterion of root elongation and 2 atm. (series A) is most favorable on the basis of dry weight of roots. When the growth of tops is taken into consideration, all the lower ratios of calcium to magnesium (Ca/Mg values from 20/80 to 0/100) are distinctly inferior to the higher ratios, and it may be provisionally concluded that solution 5 (Ca/Mg = 80/20) with a total concentration of salts equivalent to 1/2 atm. represents the best of the solutions employed in this study.

The data secured in experiment 3 serve to supplement those of the first experiment. The results were essentially the same in the two experiments. Observations demonstrated that as the calcium was reduced between 2 per cent and 0 per cent of the total concentration of calcium and magnesium there was a pronounced decrease in root elongation and likewise in the number of lateral roots that were produced. The root tips of the plants in these solutions deficient in calcium took on the same general appearance as that described previously, and the culture solutions again became cloudy at the time that small viscid droplets appeared on the root tips. The roots that were supplied with a solution containing a small quantity of boron in addition to the usual constituents did not differ from those which lacked boron. It is interesting to note that even in solution 3, in which the ratio of calcium to magnesium was only $\frac{1}{2}/99\frac{1}{2}$, there was a small increase in root length and some development of laterals, whereas root growth was entirely inhibited in the solution from which calcium was absent.

During the course of experiment 3 several attempts were made to bring about the recovery of roots that had been injured by immersion for short periods of time in complex culture solutions devoid of calcium; the injured roots were transferred to similar solutions containing calcium, and their subsequent condition was studied. But these trials were all unsuccessful. The injured roots never recovered; instead, new adventitious roots or laterals developed, which continued to grow and thus served to establish new growing apices. It became evident that even if plants were kept

for only one day in a complex culture solution from which calcium was absent, the tips of the seminal roots were permanently injured.

Other observations demonstrated that the roots were not significantly injured by immersion for relatively long periods (four to seven days) in distilled water, which of course supplied no calcium. It is evident therefore that the rapid injury which occurred when calcium was omitted from the complex culture solutions could not have been due merely to the failure of this solution to supply calcium to the roots. It must have resulted from the unbalanced condition of the culture solution, the components of which were toxic when not antagonized by calcium.

Corn. Although periodic observations were not made during the course of experiment 2, with corn, the roots at the end of twenty-five days, when fixations were made, resembled those of the wheat plants that had been grown in similar solutions. In solutions 8 and 9 of this experiment, in which the proportion of calcium to magnesium was very low, the root tips were crooked and exhibited a brownish discoloration. The lateral roots had elongated more rapidly than the primary roots and almost covered the tips of the latter. The total number of the lateral roots, however, was much smaller than in the better solutions.

Cytological effects

The cytological investigation, like the study described in the preceding section of this paper, was mainly concerned with the root tips of the plants grown in the culture solutions in which the ratio of calcium to magnesium was so low that root growth was greatly diminished. It has already been pointed out that unless the Ca/Mg ratio was reduced to a very low value, the root growth was not materially retarded. In a general way it may also be said that the cytological study, to be reported here, has shown that the cell structure of the root tips does not seem to be significantly altered except under similar conditions of relatively great calcium deficiency.

All observations recorded here were made of median sections in the apical region of the seminal roots. It is this part of the root, of course, which is fundamentally concerned with initial cell division and enlargement. Description of observations on cellular structure can best be presented by constant reference to the figures of plates 9–13.

Wheat. The results of experiments 1 and 2, on wheat, will be considered together, because of the similarity of the observations pertaining to them. In figure 1 of plate 9 there is shown a section of a young wheat root tip which had grown for four days in distilled water at the beginning of one of the experiments. This section illustrates the appearance of all root tips at

the time of immersion in the culture solutions. Nuclei and cytoplasm are normal, and active mitosis is going on. The conditions represented by this section were maintained throughout the active growth period of roots that were kept in solutions in which the calcium content was relatively high. As the activity of the root tip lessened, the merisematic region was reduced, dividing cells were less prevalent, and the cytoplasmic content was decreased, until finally the root apex became non-functional.

If the calcium content of the solution were low, but if calcium were not entirely absent (for example, with solution 1, experiment 3), the root tips, as far as the prepared slides indicated, underwent no pathological changes during the first few days, and sometimes none were apparent even after a week. Then, clear, non-stainable regions gradually made their appearance in the cytoplasm. These hyaline regions resemble vacuoles, though their mode of origin is probably quite different from that of the vacuoles which normally form in enlarging cells. They appear to arise by a process of erosion, possibly involving a hydrolytic dissolution of the cytoplasm. Finally the remaining cytoplasm came to occupy a peripheral position adjacent to the cell wall, so that the tip gave the appearance of being destitute of practically all contents except nuclei (fig. 2, pl. 9). The nuclei did not seem to be affected while these changes were taking place in the cytoplasm. A few cells from the developing metaxylem strand of the section (fig. 12, pl. 13) illustrate this condition.

In sharp contrast with this gradual loss of stainable contents is the change that occurred in cells of the root tips in solutions entirely lacking calcium (fig. 3, pl. 9). Even after two days, and at the most four, the protoplast had become badly disorganized. The cytoplasm of cells in this condition is shrunken and usually deeply stained, while the nucleus is a heterogeneous mass which cannot be differentially stained (fig. 16, pl. 13).

Regardless of whether the process was one of quick disorganization, as in the case of the roots in solutions totally devoid of calcium, or gradual, as was observed when some calcium was present, the cell contents eventually were reduced until they finally disappeared—or at least became non-stainable by the ordinary methods of cytological technique. The cytoplasm always was the first part of the cell to be affected; after it had become a neglibible quantity in the cell, a deep staining body, the former nucleus, still persisted as a heterogeneous mass. Soon thereafter the nucleus disappeared, leaving nothing visible but the cell walls. The nucleus, like the cytoplasm, thus became either totally disintegrated or so modified as to lose its attraction for cytological stains.

The cells of the epidermis and the cortex were the first to show the changes described above, and accordingly, the cells of these regions were

the first to lose their stainable contents (fig. 4, pl. 10). Later the vascular cylinder underwent the same modifications, until sometimes only the central metaxylem strand was left with its protoplasmic contents (fig. 5, pl. 10). The final result was always the same. The root tip in this stage resembled the line drawings so often found in text books (fig. 6, pl. 10). The cell walls have apparently retained their former shape, even though the cells lack their usual protoplasmic contents. It has already been noted that the roots became very brittle soon after immersion in calcium-deficient solutions; this brittleness perhaps indicates some change in the cell walls which accounts for their rigidity and prevents their collapse. The process of degeneration has occurred without any conspicuous pathological modification of cell size and form, and the arrangement of the tissues is not evidently different from that in the normal root. In fact, this method of treating roots might be recommended as a useful one for securing clear outlines of cells and tissues, unobscured by protoplasmic contents. Soon after the cell contents had disappeared from the whole apex, or in some cases earlier, fungi and bacteria began to attack and destroy the root tip. Sterile cultures would be helpful in any further study of these phenomena.

It was very interesting to observe the condition of the lateral roots, in the cultures in which they were produced, while the tips of the seminal roots that bore them were undergoing these changes. In many cases the tip of the main root became covered with a crown-like growth of apparently normal lateral roots. Numerous elongated lateral branches arose near the end of the seminal root and surpassed it in length. Sections of this material showed the main tip to be in a state of degeneration, in many cases being empty of cellular contents, and yet the lateral branches, especially the youngest, were in every way normal. Some of the older branches degenerated and later lost their contents. It seems probable that a further study over longer periods of time would reveal a series of progressive changes similar to those observed in the tips of the seminal roots.

Cells that were undergoing mitosis at the time of immersion in the low-calcium solutions seemed to be the most resistant to this process of disorganization which results in a loss of cellular contents. In the cortical region of some root tips, where the majority of the cells had lost their stainable contents, cells such as that illustrated in figure 15 of plate 13 have occasionally been observed. In this case the cells on every side had become vacant except one, and that contained only the remains of a nucleus. A cell of this sort might readily remain in the binucleated condition, if it were unable, owing to cytoplasmic disorganization, to complete the process of cell wall formation. As a result, two nuclei would be left within the confines of the mother cell wall. Binucleated cells have actually been

observed in some root tips, though they seem to be comparatively rare.

In the solutions totally lacking calcium many constricted root tips were observed. These constrictions did not seem to be confined to any particular place; in some cases they were just a few microns from the end, and in others behind the meristematic region. The cellular condition of such tips always was similar to that illustrated in figure 3 of plate 9.

Corn. The results obtained with corn were similar in all respects to those secured with wheat, in so far as the experiments with the two plants were comparable. Thus the observations on the corn confirm those on wheat, and they greatly strengthen the idea that these phenomena may be of rather general occurrence among different kinds of plants. It should be recalled that all fixations of the tips of the primary roots of corn were made at the same time, and that none of the solutions were totally devoid of calcium although some had very low concentrations of this element.

In solutions having a high concentration of calcium all tissues of the root tips presented a normal appearance (fig. 7, pl. 11), essentially like that of wheat roots in similar physiologically balanced solutions. The condition of the cellular contents was typical of that of healthy roots in general. This may be illustrated by the normal condition of the cells of one of the metaxylem strands (fig. 11, pl. 13).

On the other hand, the roots in solutions low in calcium presented a very different appearance. In an incipient stage of degeneration all of the cells of the root tip contained nuclei, although these were far from normal in appearance, and the cells were deficient in cytoplasm (fig. 8, pl. 11). A closer examination of some of the metaxylem cells of this tip shows that there remained in the cells small quantities of stainable material besides the nuclei. A more advanced phase of injury was characterized by a further loss of protoplasmic contents; the epidermal and cortical cells of a root in this condition were empty, while cells of the vascular cylinder still retained some nuclear and cytoplasmic remnants (fig. 9, pl. 12; fig. 14, pl. 13).

Finally, in solutions of very low calcium content, we may find root tips similar to those found in wheat in which the cell contents are almost entirely absent and the usual line drawing appearance is presented (fig. 10, pl. 12). In those few cells which were not empty the protoplasmic contents were limited to a much reduced nucleus (fig. 13, pl. 13).

It should be mentioned that the constriction of the root tips and the quick destruction of the protoplasts, as observed in wheat, were not seen in the experiment with corn. This is probably due to the fact that all the solutions for corn contained some calcium. Attacks of fungi and bacteria were evident after the corn roots had passed the final stage represented in figure 10 of plate 12. As in wheat, numerous lateral branches developed near

the severely injured apices of the roots; and to all appearances these branches remained normal for some time after the main apex had died.

DISCUSSION

The results reported in the preceding section of this paper demonstrate clearly that when the complex culture solutions employed in this study were deficient in calcium the cells of the meristematic region of the root tips of both wheat and corn underwent profound changes, which resulted in a marked reduction or, in many cases, a complete loss of stainable cell contents. The beginning of these changes was manifested by a gradual erosion or dissolution of the cytoplasm, and the process continued until finally, in severe cases of injury, there was a complete disappearance of the cytoplasmic contents of the cells—or at least a transformation of the cytoplasm into unstainable products. Accompanying this disintegration of the cytoplasm, but somewhat slower in its progress, was the similar change observed in the nucleus. This cellular component at first appeared to show no alteration, but later it became transformed into a disorganized body which took a dark stain and failed to exhibit any differentiated structure. Although this body persisted longer than the cytoplasmic remains, it gradually became smaller and its ultimate fate was the same as that of the cytoplasm; its final disappearance left the cell totally devoid of stainable contents.

In the early stages of the cytoplasmic changes described above, clear regions appeared within the cell. Sorokin and Sommer (1929), who observed similar changes in the cells of the root tips of *Pisum sativum*, considered that these hyaline regions were produced by a process of vacuolization. But it seems doubtful whether the change observed in the root tips of the present study should be regarded as comparable to the true vacuolization which occurs when the normal cell is enlarging during the absorption of water. The meristematic cells were not increasing in volume during the formation of the hyaline regions. The boundaries between these regions and the cytoplasm were more irregular than those of normal vacuoles. Moreover, the hyaline regions continued to develop progressively while the stainable contents simultaneously diminished. In the last stages before the complete disappearance of the cell contents, small portions of cytoplasm were left irregularly distributed in various parts of the cell. The large clear area of the cell at this time in many cases extended to parts of the cell wall and bore little or no resemblance to a normal vacuole.

The changes here involved seem to indicate some process of erosion which disintegrates the protoplast. The process is suggestive of a hydrolytic dissolution resembling autolysis. Thus the hyaline regions left in the

cell probably contain at first the dissolved products of the protoplasm, rather than the usual constituents of the cell sap that would be in vacuoles. Later they may contain only water, since the cell wall is generally readily permeable to solutes and it may be supposed that these dissolved materials gradually diffuse out of the cells—into the surrounding culture medium and possibly into other portions of the roots.

Death of the cells undoubtedly occurred before the final disappearance of stainable contents. This was evidenced by the failure of cells exhibiting incipient injury to recover after the roots had been transferred to physiologically balanced solutions. Death probably took place at a rather early stage in the process of cytoplasmic disintegration, although further study would be needed to determine the death point with any high degree of precision. Tests of plasmolysis and recovery and of the entrance of stains to which living cells are impermeable might be useful in this connection.

The occurrence of pronounced injury and consequent protoplasmic disintegration appeared to be definitely related to the calcium content of the culture solutions employed in this study. The injury was limited to a critical range of Ca/Mg ratios which lay between 0/100 and 5/95. In these toxic solutions the calcium salt made up from 0 to 2.5 per cent of the total molecular concentration of all the salts. The critical range was the same for series A of experiment 1 as for series C, although the actual concentrations of calcium in series A were sixteen times as great as in series C. This furnishes convincing evidence that the ratio of calcium to magnesium, or the ratio of calcium to all of the other components of the solution, rather than the absolute concentration of calcium, was the factor which determined the occurrence of injury. The rate and severity of injury in the toxic solutions were inversely proportional to the ratio of calcium to magnesium, or to the ratio of calcium to the other constituents of the solution. With the solutions lacking calcium the action was such that permanent injury occurred soon after the immersion of the roots; but when small quantities of calcium were present, the injury was delayed for a period of time which depended upon the calcium content of the solution. These conclusions are based on the cytological appearance of root sections, the rate of growth, and the final length and dry weight of the seminal roots.

When calcium was entirely absent from the complex culture solutions, root growth ceased almost immediately and the series of degeneration changes within the cells progressed rapidly. But in distilled water, which of course lacks calcium, the growth of seedling roots continued for a number of days. Such roots elongated considerably and produced lateral branches. While this growth was taking place, the cellular condition of the root apices exhibited no significant abnormalities. If calcium is assumed to

be a necessary cellular constituent, we must suppose that sufficient quantities of this element for considerable growth of the seminal roots were derived from the material stored within the seed. The immediate cessation of root growth in the complex solutions lacking calcium must therefore be attributed to the physiologically unbalanced nature of these solutions, rather than to their failure to supply calcium. In the absence of calcium the other constituents of the culture solution, especially magnesium, were present in sufficient concentration to be poisonous to wheat roots (Trelease and Trelease, 1926a).

Although the present experiments were planned with reference to variations in the Ca/Mg ratios of the solutions, it should be emphasized that the influence of these solutions on the plants undoubtedly depended upon the complex balance of the culture solutions. If other basic solutions had been employed, the relations of the plants to the Ca/Mg ratios might have been quite different from those obtained in this study. A more nearly complete study would of course involve the use of many more culture solutions, in which all components were varied systematically. Throughout this paper the results have been discussed in their relations to the ratio of calcium to magnesium in the solutions that were used. Considering the manner in which the solution components were varied, the results might be interpreted in more general terms with reference to the ratio of calcium to all of the other constituents of the solutions.

The disintegration of the cell contents that occurred in root tips subjected to toxic solutions must have resulted in soluble products that were able to diffuse out of the injured cells, presumably into the culture solution as well as into other cells of the root. With the dissolution of the protoplast, particularly the cytoplasm, the semi-permeability of the cell must have been destroyed, thus releasing the various soluble materials derived from the former protoplast.

The bearing of this process on the numerous studies of exosmosis, leaching, and excretion, as the phenomena have been variously termed in the literature, is obvious. Probably many of the extreme cases of exosmosis are not due to changes in permeability of living cells, but rather to postmortal changes and excretions due to the destruction of the protoplast of the cell. This might be especially true where anesthetics, acids, etc., have been used to increase the permeability or where nutrient solutions lacking calcium have been employed (True, 1916, Iljin, 1928), thus allowing the various constituents of the solutions to be poisonous. Osterhout (1923), by means of electrical resistance measurements of the protoplast, has shown this to be true in the case of *Nitella* when chloroform is applied.

It is recognized that many studies have shown that exosmosis occurs

even when roots are immersed in distilled water. In fact, the excretion of materials from roots has been used to demonstrate the so-called toxicity of distilled water. It has also been shown that low concentrations of a calcium salt have an inhibitory effect on such excretion. But even more rapid exosmosis than that occurring in distilled water has been found to take place when unbalanced solutions, lacking calcium, are employed. Cases of this kind may probably be explained as due to an actual destruction of the protoplasts, such as was observed in the present study.

The work of Wächter (1905), Hansteen Cranner (1910, 1914, 1919, 1922, 1926), True (1914, 1915a, 1915b, 1916), Merrill (1915a, 1915b), Hibbard (1915), Stiles and Jorgensen (1915), Brooks (1916), Iljin (1928), Steward (1928), and many others has shown that the products of exosmosis, under a variety of conditions and with various kinds of tissues as well as with roots, may be electrolytes, sugars, lipoids, etc. If such a disorganization of the protoplast as that described here occurs in some of these cases, we should expect to find the soluble forms of all the substances which go to make up an aggregate protoplasm. It is strange that protein seems to be lacking in the exudates from plant cells undergoing exosmosis; at least it has never been reported. Although the protoplasm includes a variety of proteins, it is possible that the proteins are completely converted into soluble products, such as amino acids, and thus the culture solutions containing the exuded products may not give the ordinary tests for protein.

The turbidity observed in the culture solutions during the course of these experiments did not seem to coincide with the time of the disorganization of the protoplasm. It did not become conspicuous until several days after the cell changes had taken place. This suggests that the action of bacteria and fungi on the disorganized roots or on their exuded products may have been the causal factor. Wächter (1905), Merrill (1915a), and Steward (1928) have reported that the solutions did not become turbid if precautions were taken to sterilize them. On the other hand, Hansteen Cranner (1922) concluded that the turbidity observed in his cultures was due to the presence of insoluble lipoids derived from the protoplast, especially from the part which he regarded as infiltrating the cell wall and being responsible for the maintenance of normal permeability in the cell. He considered that their loss involved a destruction of this condition of permeability.

Certain effects of calcium deficiency on the meristematic regions of the higher plants have been previously reported by Reed (1907) in the root tips of *Zea mays*, Warthiadi (1911) in the apical meristem of *Tradescantia*, and Sorokin and Sommer (1929) on the root tips of *Pisum sativum*. They all report cell changes, but not the complete disintegration of the

protoplasm that was observed in this investigation. Since the meristematic regions of the root apex seem to be the first parts affected, it is probable that many cases of dwarfed plants reported in the literature can be explained as due to injury to these parts resulting from calcium-deficient solutions. Less severe injury may not prevent the formation of mature tissues. Thus, it has been reported by Day (1929) that the mature regions of the root were normal, even though the plants were dwarfed. She did not investigate the root tips.

The failure of the cell walls to collapse after the destruction of the protoplast in the injured tips suggests that some change was induced in these structures as well as in the cell contents. The nature of this change could only be ascertained by chemical tests, since microscopic examination revealed no visible modification of the walls. Albrecht and Davis (1929) found difficulty in preparing sections of soy-bean stems when the plants had suffered from calcium starvation, and they suggested that this indicated some change in the structure of the cell walls.

SUMMARY

The influence of calcium-deficient solutions upon the growth and cytological structure of the root tips of young wheat and corn seedlings is reported in this paper.

In such solutions the cells of the root apex gradually lost all their stainable contents. The cell walls remained intact, so that the final appearance of the meristematic root apex was comparable to that of a line drawing in a text book.

The cytoplasm was affected first. It seemed to undergo a gradual erosion, suggestive of hydrolytic dissolution, until it completely disappeared. During the early changes in the cytoplasm the nucleus remained normal but eventually it degenerated into a heterogeneous mass and finally disappeared.

This process of protoplasmic disintegration first affected only the peripheral layers of the cells in the epidermal and cortical regions. Later it progressed to the interior and finally upward in the roots.

It seems probable that many of the extreme cases of exosmosis reported in the literature, especially those which follow treatment with toxic agents, are due to protoplasmic disintegration, rather than merely to changes in the permeability of living cells.

In the present experiments the occurrence of pronounced injury was correlated with the ratio of calcium to magnesium in the culture solution, or with the ratio of calcium to all the other components of the solution. It was independent of the absolute concentration of calcium. Marked in-

jury, resulting in a total loss of cell contents, did not occur when the Ca/Mg ratio was 5/95 or higher, or when the concentration of the calcium salt was 2.5 per cent or more of the total molecular concentration of the solution.

Growth ceased almost immediately after immersion of a root in a complex culture solution from which calcium was excluded. Through a rather definite critical range the rate of root growth was roughly proportional to the calcium content of the solution or to the ratio of calcium to magnesium.

No lateral roots developed when calcium was entirely absent from the culture solution. When small amounts were present, the lateral roots continued to elongate after growth of the main root had ceased.

The pronounced injury that occurred in complex solutions deficient in calcium appeared to be due to the physiologically unbalanced nature of these solutions. In the absence of sufficient calcium, the other constituents of the culture solution, especially magnesium, were highly toxic to the cells of the roots.

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Explanation of plates

The photomicrographs were taken with the aid of a Bausch & Lomb 16 mm. objective and a 7.5× compensating ocular, the resulting magnification being 140×. The figures in plate 5 were drawn with a camera lucida, using a Zeiss 2 mm. apochromatic objective and a 10× compensating ocular. The resulting magnification was about 1400×.

Plate 9

Median longitudinal sections of wheat root tips after:

Fig. 1. Four days in distilled water. Representative of the condition of all root tips at the time of immersion in the culture solutions. Roots in solutions of high calcium content retained this normal appearance throughout the active growth period of the terminal roots.

Fig. 2. Seven days in a culture solution with a calcium-magnesium ratio of 2/98. There is a marked reduction of cytoplasm in the cells, but the nuclei are not markedly affected thus far.

Fig. 3. Two days in a culture solution with a calcium-magnesium ratio of 0/100. The complete absence of calcium was always associated with rapid shrinkage and disintegration of the protoplasts.

PLATE 10

Median longitudinal sections of wheat root tips after:

Fig. 4. Two weeks in a culture solution with a calcium-magnesium ratio of 2/98. The cells of the cortical and epidermal regions of the root have lost their stainable contents.

Fig. 5. One week in a culture solution with a calcium-magnesium ratio of 0/100. The only cells retaining their cytoplasmic contents are those of the developing central metaxylem strand.

Fig. 6. Two weeks in a culture solution with a calcium-magnesium ratio of 2/98. This condition is representative of the condition finally reached by all the roots in solutions very low in calcium. Cell contents have entirely disappeared.

PLATE 11

Median longitudinal sections of corn root tips after 25 days:

Fig. 7. In a culture solution with a high calcium-magnesium ratio (63.6/36.4). Presents normal appearance of healthy root.

Fig. 8. In a culture solution with a much lower calcium-magnesium ratio (3.5/96.5) The cytoplasmic contents of the cells are greatly reduced, although each cell exhibits the remains of a nucleus.

PLATE 12

Median longitudinal sections of corn root tips after 25 days:

Fig. 9. In a culture solution with a very low calcium-magnesium ratio (2/98). The cells of the epidermis and cortex are devoid of protoplasmic contents.

Fig. 10. In the same culture solution as the root tip of figure 9. Nearly all the cells are devoid of protoplasmic contents.

PLATE 13

Fig. 11. Cells from a metaxylem strand of the root tip of corn illustrated in figure 7.

Fig. 12. Cells from the developing metaxylem strand of the wheat root tip represented in figure 2. The nuclei appear normal, but erosion of the cytoplasm has left a hyaline region in the cell.

Fig. 13. Cells from a metaxylem strand of the root tip of corn shown in figure 10. The majority of the cells in this section were empty, and this drawing illustrates the last stage before the complete disappearance of the contents.

Fig. 14. From the root tip of figure 9, showing the complete disintegration of the protoplast. The hyaline regions in these cells are not typical of vacuoles.

Fig. 15. A cell from the cortical region of a root tip that has almost completed division. All the surrounding cells were empty with the one exception which is figured. The persistance of cytoplasm in a cell undergoing mitosis is significant.

Fig. 16. Cells from a metaxylem strand of a root tip illustrated in figure 3. This shrunken and degenerate condition is typical of the cells of roots that had been placed for a short time in a solution destitute of calcium.

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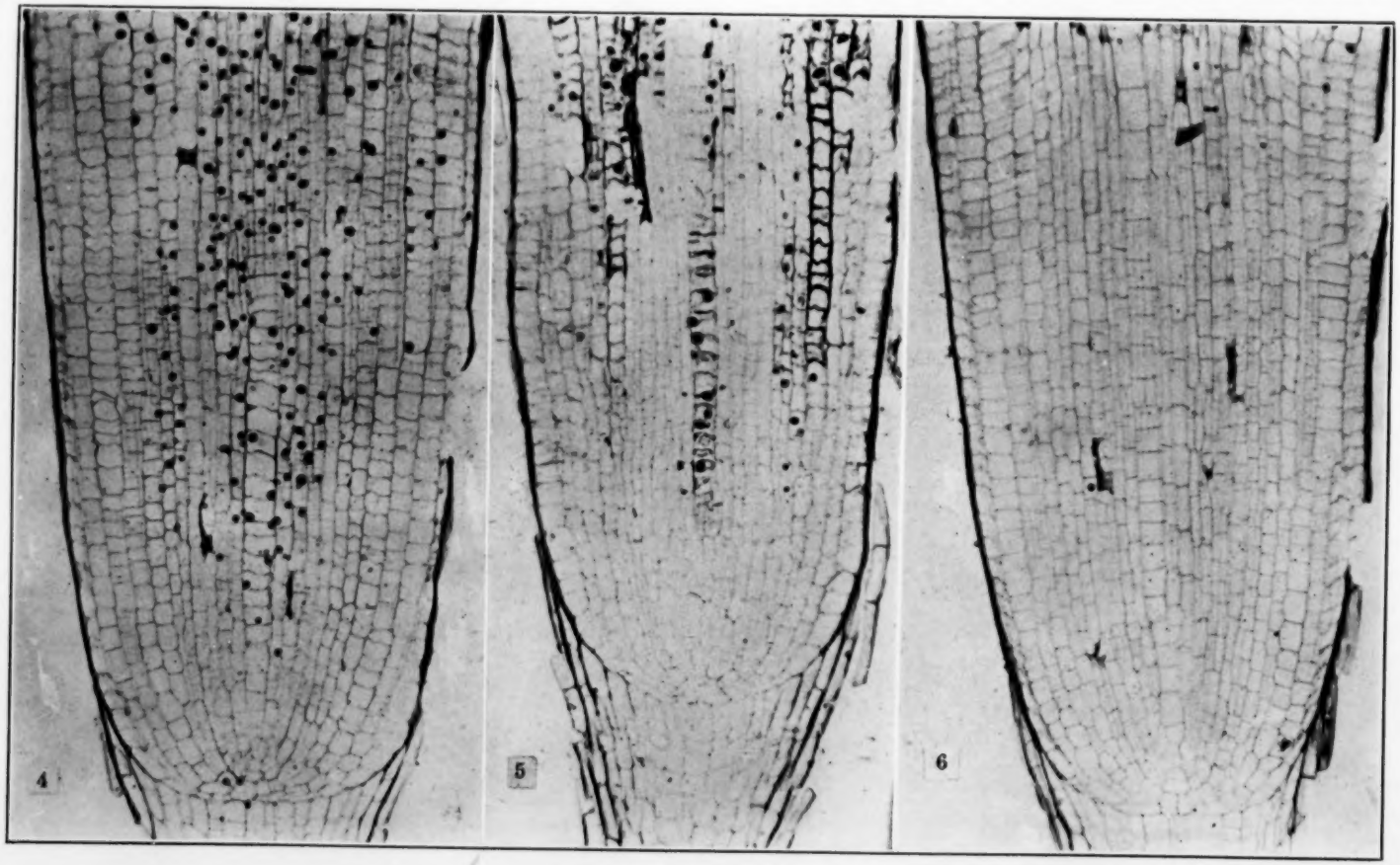

BAMFORD: CHANGES IN ROOT TIPS

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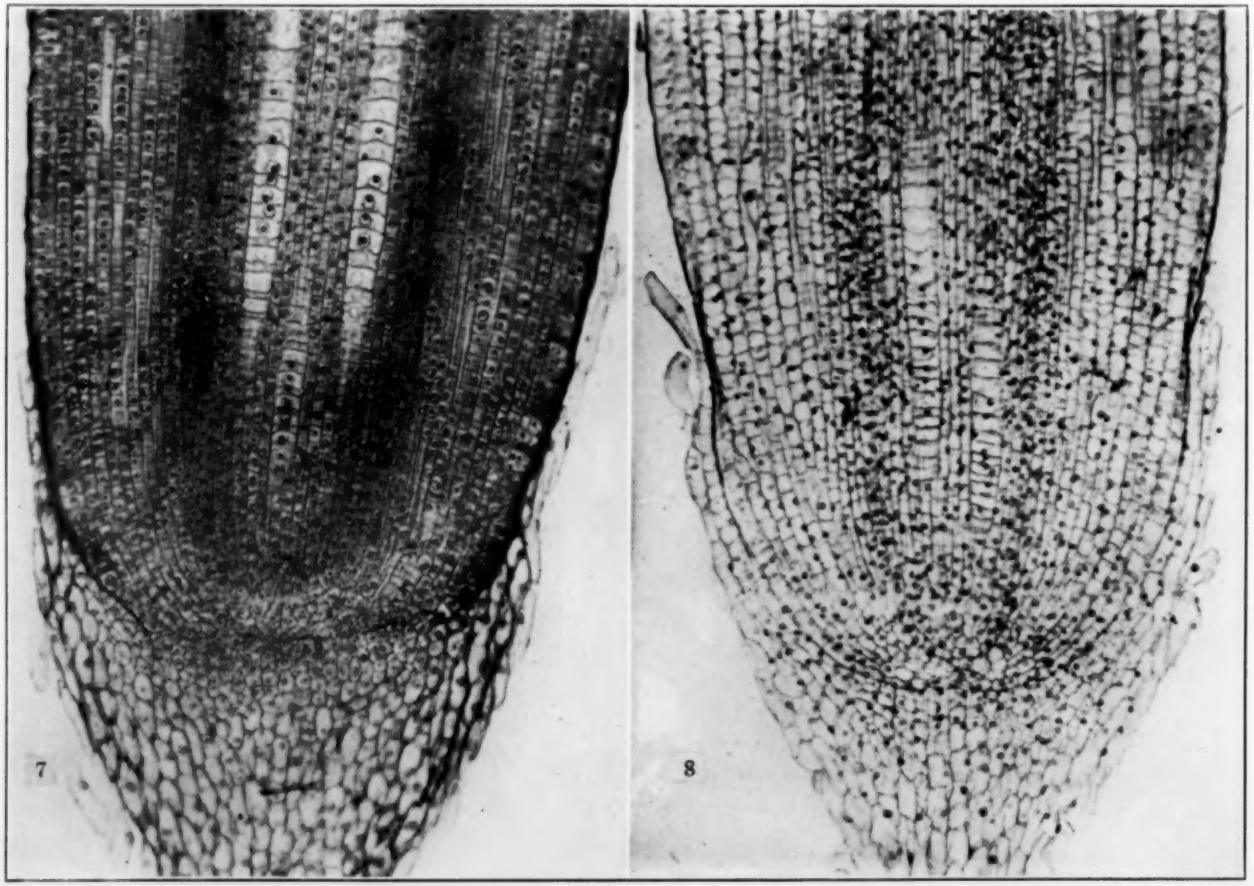

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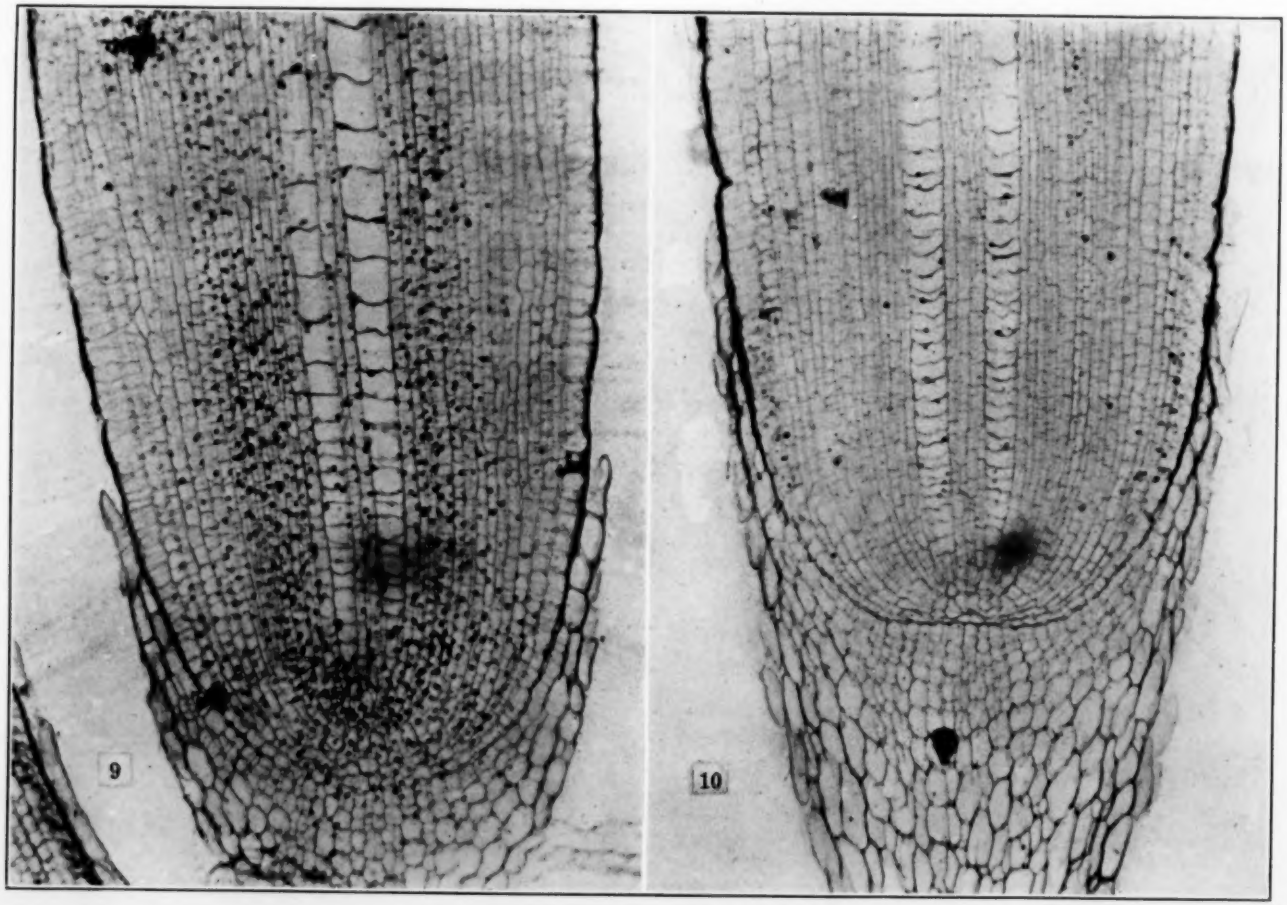

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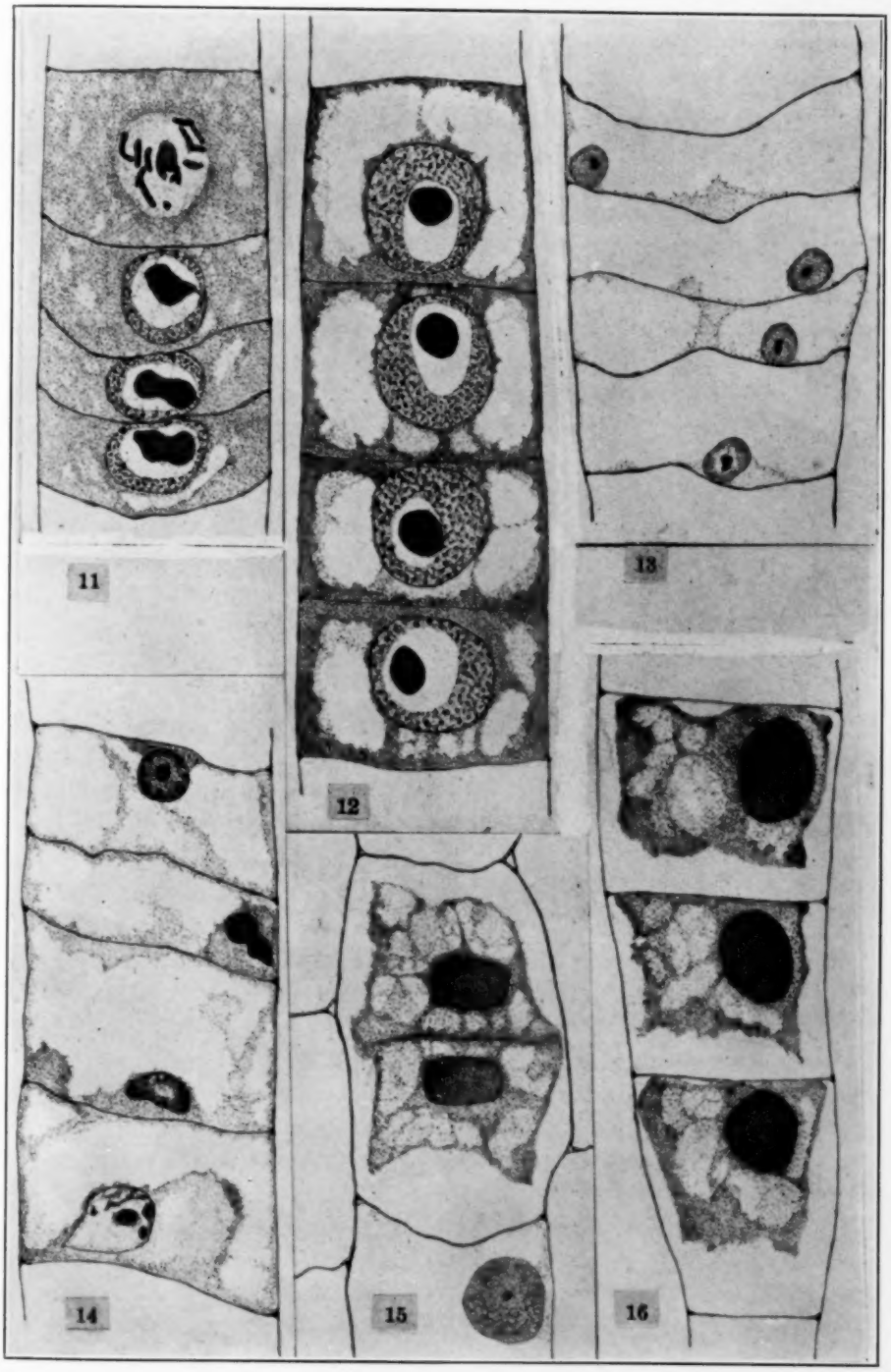

BAMFORD: CHANGES IN ROOT TIPS

Growth and variability of wheat seedlings in magnesium sulphate solutions

JOSEPH CARMIN

(WITH THREE TEXT FIGURES)

The method used in this work was essentially the one described by Trelease and Trelease (1925). The wheat grains (pure line of spring wheat, Marquis, Saskatchewan, no. 70, selection no. 313, grown during the summer of 1922 and supplied by the University of Saskatchewan through the kindness of Professor Manley Champlin) were soaked for three hours in distilled water. Selected seeds were then distributed uniformly, furrow-side down, in glass moisture chambers containing two layers of white blotting paper saturated with distilled water. The germinators were placed in a dark chamber in which the temperature was kept fairly constant. The blotting paper was moistened with distilled water once or twice during germination. The seeds were left there about 24 hours, or until the primary root had attained a length of about 5 mm. They were then transferred to the culture vessels.

Each culture vessel consisted of a glass tumbler with a capacity of about 275 cc. placed in a larger beaker (600 cc.). The mouth of the smaller vessel was covered with a piece of a paraffined bobbinet tied with a paraffined linen thread. The vessels were filled with the culture solution until the level of the solution in both of them was even with the top of the smaller vessel.

The culture solutions were made up of Powers-Weightman-Rosengarten's analytical chemicals. The label for magnesium sulphate ($MgSO_4 \cdot 7H_2O$) showed the following analysis in percentages: alkali sulphate, 0.050; arsenic (As), 0.0000; calcium (Ca) 0.00; heavy metals 0.0000; iron (Fe) 0.0005; chloride (Cl) 0.0007. Twenty-five germinating seeds which were nearly uniform in appearance were selected and put on the netting of each culture vessel. The cultures were kept under the same conditions as the germinators. The first two days they were covered with an inverted watch glass. With each experiment two control cultures were grown in a balanced solution which contained 0.002 M $MgSO_4$, 0.002 M KH_2PO_4, and 0.002 M $Ca(NO_3)_2$. In addition, a single culture was grown in distilled water obtained from a Barnstead still. Each experiment was terminated when the longest root in the balanced solution had attained a length of about 90 mm. The seedlings of each culture were placed in a killing solution containing 0.2 per cent chromic acid and 0.1 per cent acetic acid dissolved in distilled

water. This solution proved to have no contracting influence upon the roots and tops of the seedlings.

Concentrations ranging from 0.0005 M to 0.0150 M $MgSO_4$ were tried. None of them showed any precipitate. For each solution one hundred seedlings were grown, twenty-five in each of four culture vessels. Two of the experiments were carried on simultaneously and the other two individually. The first and second experiments were carried out at a temperature of 17°–20° C., and it took 117 hours before the longest root in the balanced solution attained the required length (of approximately 90 mm.). The third experiment was carried out at 13°–22° C. for 130 hours, and the fourth experiment at 11.5°–20° C. for 150 hours. The low temperatures occurred only for short periods of time. The results show just as close an agreement between the first, third, and fourth experiments, which were carried on separately, as between the first and second one, carried on simultaneously. This indicates that the differences in temperature and other environmental conditions were not large enough to influence significantly the validity of the results of the experiments.

Four measurements were recorded for each seedling: the length of the principal root, the lengths of the two longest lateral roots (one from each side), the length of the longest root, and the length of the top. The results of the measurements for each lot of twenty-five seedlings were averaged and recorded. The probable error (P.E.) was computed in two ways: by the usual formula, and also as percentage of the mean, which gives a better idea of the discrepancies in probable error between the different experiments. The four experiments were then compared using Rietz and Smith's (1910) method. All cases were discarded in which the probable error of the difference between the experiments calculated by this formula was higher than five times the probable error. The remaining data were then averaged and plotted. Top root ratios were also calculated. The variability coefficients (C) were calculated by the usual formula ($C = 100\ \sigma$ divided by M) and plotted. The probable error of the variability coefficient was calculated by the formula: $E_c = 0.6745\ \sigma$ divided by $\sqrt{2n}$, and also as percentage of the variability coefficient. In a few cases the variability curves for the different organs in the different solutions used were also plotted. The skewness of the variability curves was calculated by Pearson's formula: S = Mean minus Mode divided by σ. Finally the correlations between the different organs of the plant as well as between the left and the right side of the plant were calculated for the various solutions used.

RESULTS

The only morphological modification observed was that the tips of the roots became greatly swollen in the higher concentrations of the salt. The

growth of the roots in distilled water was almost as good as that in the balanced solution, as can be seen from tables 1–4 and figure 1, while the

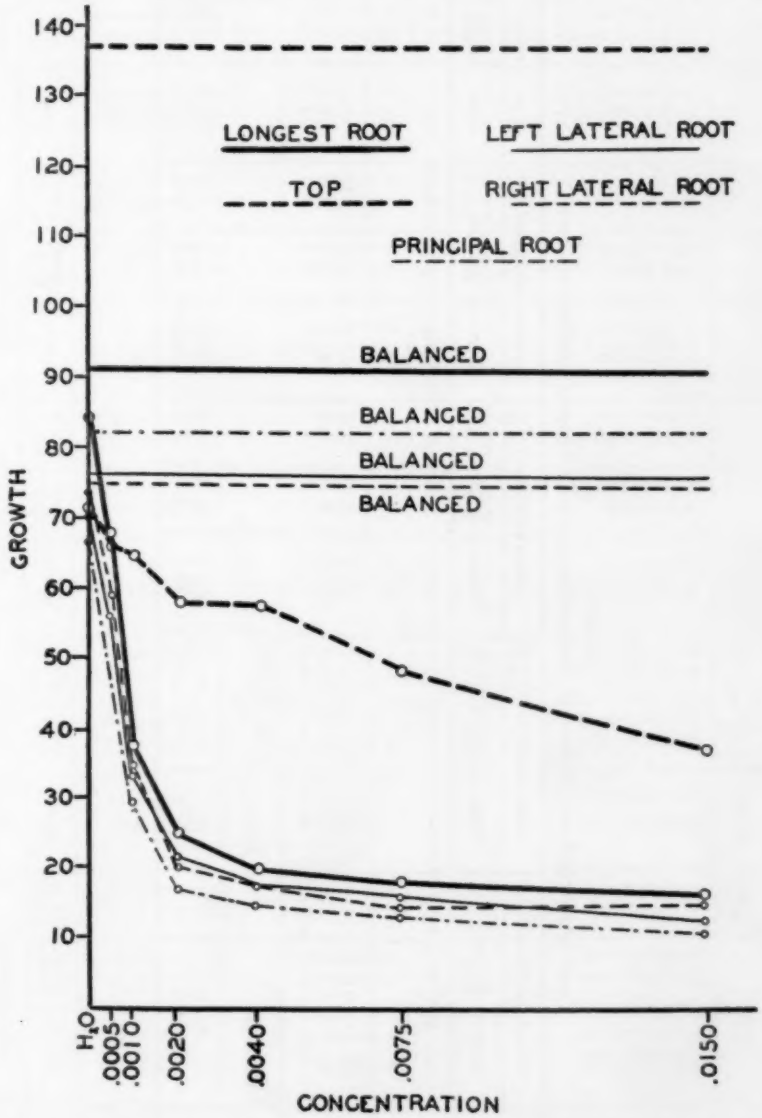

Fig. 1. Length of roots and tops (in mm.) of wheat seedlings grown in magnesium sulphate solutions.

tops attained in distilled water a length equaling approximately 50 per cent of their growth in the balanced solution. This indicates that distilled water lacks one of the elements necessary for normal top growth in young

TABLE 1

Length of principal roots (in mm.) of wheat seedlings grown in magnesium sulphate solutions

CONCENTRATION	FIRST EXPERIMENT			SECOND EXPERIMENT			THIRD EXPERIMENT			FOURTH EXPERIMENT			AVERAGE		
	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M
Dist. Water	—	—	—	—	—	—	61.1	4.1	6.7	72.2	2.1	2.8	66.6	2.5	3.7
0.0005M	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.0010M	27.2	1.2	4.4	—	—	—	30.0	2.1	7.0	29.2	0.7	2.4	28.8	0.8	2.8
0.0020M	15.9	0.6	3.8	15.9	0.8	5.0	—	—	—	18.1	0.5	2.8	16.6	0.4	2.4
0.0040M	14.2	0.7	4.9	14.8	0.7	4.7	16.9	0.7	4.1	15.4	0.5	3.2	15.4	0.3	1.9
0.0075M	12.8	0.5	3.9	12.0	0.4	3.3	14.2	0.6	4.2	12.1	0.4	3.3	12.8	0.3	2.3
0.0150M	9.0	0.3	3.3	8.6	0.3	3.5	—	—	—	9.6	0.3	3.1	9.6	0.2	2.1
	Mean		Prob. Error		P.E. in % of M										
Balanced	86.0 76.1		1.6 2.2		1.9 2.9		84.7 74.7	4.1 3.9	4.8 5.2	83.9 84.6	0.9 0.9	1.1 1.1	81.6	1.1	1.3

TABLE 2

Length of left lateral roots (in mm.) of wheat seedlings grown in magnesium sulphate solutions

CONCENTRATION	FIRST EXPERIMENT			SECOND EXPERIMENT			THIRD EXPERIMENT			FOURTH EXPERIMENT			AVERAGE		
	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M
Dist. Water	—	—	—	—	—	—	66.1	3.4	5.1	76.6	1.3	1.7	71.4	2.0	2.8
0.0005M	60.2	1.6	2.6	51.8	2.7	5.2	—	—	—	—	—	—	56.0	2.1	3.8
0.0010M	38.3	1.2	3.1	30.3	1.4	4.6	31.8	1.5	4.7	32.3	0.9	2.8	33.2	0.7	2.1
0.0020M	19.8	0.9	4.5	22.6	1.0	4.4	20.3	0.7	3.4	20.4	0.6	2.9	20.8	0.4	1.9
0.0040M	16.3	0.7	4.3	18.5	0.7	3.8	16.7	0.9	5.3	16.5	0.6	3.6	17.0	0.4	2.4
0.0075M	13.8	0.5	3.6	13.7	0.5	3.6	15.4	0.6	3.9	15.8	0.6	3.8	14.7	0.3	2.0
0.0150M	10.8	0.5	4.6	11.2	0.5	4.5	—	—	—	11.8	0.5	4.2	11.3	0.3	2.6
	Mean		Prob. Error		P.E. in % of M										
Balanced	71.5 75.3		2.9 1.1		4.1 4.3		69.4 77.4	2.9 2.6	4.5 3.5	80.1 81.1	0.9 0.8	1.1 1.0	75.8	0.9	1.2

TABLE 3

Length of right lateral roots (in mm.) of wheat seedlings grown in magnesium sulphate solutions

CONCENTRATION	FIRST EXPERIMENT			SECOND EXPERIMENT			THIRD EXPERIMENT			FOURTH EXPERIMENT			AVERAGE		
	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M
Dist. Water	—	—	—	—	—	—	65.1	2.6	4.0	80.5	1.4	1.7	72.8	1.9	2.6
0.0005M	63.0	1.6	2.5	54.7	2.8	5.1	—	—	—	—	—	—	58.9	1.7	2.9
0.0010M	40.3	1.3	3.2	28.2	0.3	3.2	35.0	1.5	4.3	32.6	1.2	3.7	34.0	0.7	2.1
0.0020M	20.5	0.7	3.4	20.2	0.9	3.0	17.5	1.0	5.7	20.5	0.7	3.4	19.7	0.4	2.0
0.0040M	15.3	0.6	3.9	16.2	0.5	3.1	17.8	0.7	4.5	16.4	0.7	4.2	16.5	0.4	2.4
0.0075M	15.2	0.5	3.2	12.5	0.5	4.0	13.9	0.6	4.3	15.4	0.6	3.9	14.2	0.3	2.1
0.0150M	11.8	0.5	4.2	12.0	0.5	4.2	14.9	0.5	3.3	13.1	0.9	6.9	12.9	0.3	2.3
	Mean		Prob. Error		P.E. in % of M										
Balanced	75.5 71.8		2.6 2.6		3.4 3.6		63.5 74.8	3.3 2.0	5.2 2.7	81.3 82.1	1.0 0.7	1.2 0.8	74.8	0.9	1.2

TABLE 4

Length of longest roots (in mm.) of wheat seedlings grown in magnesium sulphate solutions

CONCENTRATION	FIRST EXPERIMENT			SECOND EXPERIMENT			THIRD EXPERIMENT			FOURTH EXPERIMENT			AVERAGE		
	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M
Dist. Water	—	—	—	—	—	—	82.8	1.7	2.1	84.6	1.0	1.2	83.7	1.0	1.2
0.0005M	68.2	1.3	1.9	67.3	1.9	2.8	—	—	—	—	—	—	67.7	1.1	1.6
0.0010M	—	—	—	33.5	1.3	3.9	42.1	1.4	3.3	36.1	0.9	2.5	37.2	0.8	2.2
0.0020M	23.5	0.6	2.5	25.0	0.9	3.6	28.0	0.8	2.8	23.2	0.7	3.0	24.9	0.4	1.6
0.0040M	19.2	0.5	2.6	20.3	0.6	2.9	21.1	0.7	3.3	19.7	0.5	2.5	20.0	0.3	1.5
0.0075M	16.9	0.4	2.4	14.9	0.5	3.3	17.8	0.5	2.8	17.7	0.6	3.4	16.8	0.3	1.8
0.0150M	13.1	0.5	3.8	13.7	0.5	3.6	—	—	—	14.8	0.8	5.4	13.9	0.3	2.2
	Mean		Prob. Error		P.E. in % of M										
Balanced	90.8		0.9		1.0		95.3	1.6	1.7	86.1	0.8	0.9			
	—		—		—		93.0	1.2	1.3	—	—	—	91.3	0.6	0.6

TABLE 5

Length of tops (in mm.) of wheat seedlings grown in magnesium sulphate solutions

CONCENTRATION	FIRST EXPERIMENT			SECOND EXPERIMENT			THIRD EXPERIMENT			FOURTH EXPERIMENT			AVERAGE		
	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M	Mean	Prob. Error	P.E. in % of M
Dist. Water	NOT MEASURED						72.7	1.9	2.6	70.2	1.0	1.8	71.4	1.1	1.5
0.0005M							67.5	1.2	1.8	66.9	1.0	1.5	67.2	0.8	1.2
0.0010M							64.1	1.4	2.2	64.4	1.2	1.8	64.2	0.9	1.4
0.0020M							55.6	1.9	3.4	58.5	1.1	1.9	57.0	1.1	1.9
0.0040M							57.9	1.6	2.7	56.5	1.0	1.8	57.2	1.0	2.7
0.0075M							46.9	1.7	3.6	49.4	1.1	2.2	48.2	1.0	2.1
0.0150M							39.9	1.6	4.0	33.0	0.8	2.4	36.5	1.0	2.7
Balanced							146.5	1.2	0.8	125.0	0.8	0.7	136.5	3.1	2.3
							146.2	2.0	1.4	128.5	0.6	0.4			

seedlings. In figure 1 it can be seen that the curves of root growth in various concentrations of magnesium sulphate solutions are smooth, with no indication of irregularities; they fall very rapidly at first, then more and more slowly, so that finally they become practically straight lines nearly parallel to the base line, which would correspond to the so-called death point. This point agrees very well with Harter's (1905) results. The means for the length of the left and right lateral roots are approximately the same in all solutions tried, including the distilled water and balanced solutions. The principal root was about five mm. longer than the other roots in the balanced solution. This might be expected since, at the start of the experiment, it was about five mm. long, before the appearance of the lateral roots. It is interesting that, in spite of this fact, the principal root is shorter than the laterals in all magnesium sulphate solutions and distilled water as well, which means that these solutions are in some way or other more toxic to the principal roots than to the lateral ones.

The curve for the rate of top growth does not run so smoothly as those for root growth. This might possibly be explained by the fact that the averages for the tops are only for 50 individuals, while for the root values 100 individuals were measured in each case. The curve for top growth falls much more slowly than those for root growth. It seems that the roots may have had some ability to hinder the toxic magnesium sulphate from getting at the tops, either by retaining it, or in some other way. In the lowest concentrations the tops are somewhat shorter than the roots, but with increase of concentration the roots are shorter than the tops. Magnesium sulphate must hinder the growth of roots much more than that of the tops. This can also be seen from the gradual rise in the top-root ratio, which is 0.8 for distilled water; 1.0 for 0.0005 M solution; 1.7 for 0.0010 M solution; 2.3 for 0.0020 M solution; 2.9 for 0.0040 M solution; 2.9 for 0.0075 M solution; 2.6 for 0.150 M solution. For the balanced solution it is 1.5.

For all magnesium sulphate solutions there was a lack of correlation between principal root and top, between longest root and top, and between the right lateral and the left lateral root. The balanced solution showed a slight correlation between longest root and top as well as between the principal root and top, it was 0.3431 ± 0.0687 in the first case and 0.3389 ± 0.0597 in the second case. Thus under normal conditions there exists in young seedlings some correlation between growth rate of roots and tops. This correlation is broken down in some way in distilled water and in magnesium sulphate solutions.

Tables 6 and 7 contain the variability coefficients (C) for roots and tops. The probable error in percentages of C is a fairly constant number, around 10, which shows that the seeds for this experiment were selected very care-

TABLE 6

Coefficient of variability (C) for longest roots

CONCENTRATION	FIRST EXPERIMENT			SECOND EXPERIMENT			THIRD EXPERIMENT			FOURTH EXPERIMENT			AVERAGE		
	Coef. of Var.	Prob. Error	P.E. in % of C	Coef. of Var.	Prob. Error	P.E. in % of C	Coef. of Var.	Prob. Error	P.E. in % of C	Coef. of Var.	Prob. Error	P.E. in % of C	Coef. of Var.	Prob. Error	P.E. in % of C
Dist. Water	—	—	—	—	—	—	15.6	1.5	9.6	9.0	1.1	12.2	12.8	0.8	6.2
0.0005M	14.1	1.4	10.0	21.1	2.2	10.0	—	—	—	—	—	—	17.9	1.3	7.3
0.0010M	—	—	—	28.4	3.1	10.9	24.0	2.5	10.4	19.2	2.0	10.4	28.3	1.8	6.4
0.0020M	20.9	2.1	10.0	26.4	2.9	10.9	21.1	2.2	10.4	22.5	2.4	10.7	25.2	1.4	5.5
0.0040M	20.3	2.1	10.3	20.2	2.1	10.4	24.2	2.5	10.3	21.0	2.1	10.0	22.6	0.8	3.6
0.0075M	18.5	1.9	10.3	22.8	2.4	10.5	23.0	2.4	10.4	24.8	2.6	10.4	28.6	1.6	5.6
0.0150M	26.0	2.9	11.2	24.8	2.7	10.9	—	—	—	39.7	5.0	12.6	31.3	1.9	6.1
	Coef. of Var.		Prob. Error		P.E. in % of C										
Balanced	7.5		0.7		9.3		12.2	1.2	9.8	7.0	0.7	10.0	10.2	0.5	4.9
	—		—		—		10.0	1.0	10.0	—	—	—			

TABLE 7

Coefficient of variability (C) for tops.

CONCENTRATION	FIRST EXPERIMENT			SECOND EXPERIMENT			THIRD EXPERIMENT			FOURTH EXPERIMENT			AVERAGE		
	Coef. of Var.	Prob. Error	P.E. in % of C	Coef. of Var.	Prob. Error	P.E. in % of C	Coef. of Var.	Prob. Error	P.E. in % of C	Coef. of Var.	Prob. Error	P.E. in % of C	Coef. of Var.	Prob. Error	P.E. in % of C
Dist. Water	NOT MEASURED						19.7	1.9	9.6	10.7	1.0	9.3	16.1	1.1	6.9
0.0005M							13.3	1.3	9.8	10.6	1.0	9.4	12.2	0.8	6.5
0.0010M							15.9	1.6	10.0	14.3	1.4	9.8	15.1	1.1	7.3
0.0020M							24.8	2.7	10.9	14.5	1.4	9.6	20.5	1.5	7.3
0.0040M							21.9	2.2	10.0	13.6	1.4	10.3	18.5	1.3	7.0
0.0075M							26.4	1.2	4.5	17.0	1.7	10.0	22.6	1.7	7.5
0.0150M							29.8	3.4	11.4	17.6	1.8	10.2	28.3	2.2	7.8
Balanced							6.1	0.6	9.8	4.7	0.4	8.5			
							10.1	1.0	9.9	3.3	0.3	9.1	12.2	0.8	6.5

fully. The averages for variability coefficients of the longest roots and tops are plotted in figure 2. The curve for longest roots runs parallel to the one for tops. This graph shows that the solution in which the seeds are grown has a very pronounced influence upon the variability of seedling growth. The variability coefficient is the lowest in distilled water and in the balanced solution. These media might be regarded as furnishing natural con-

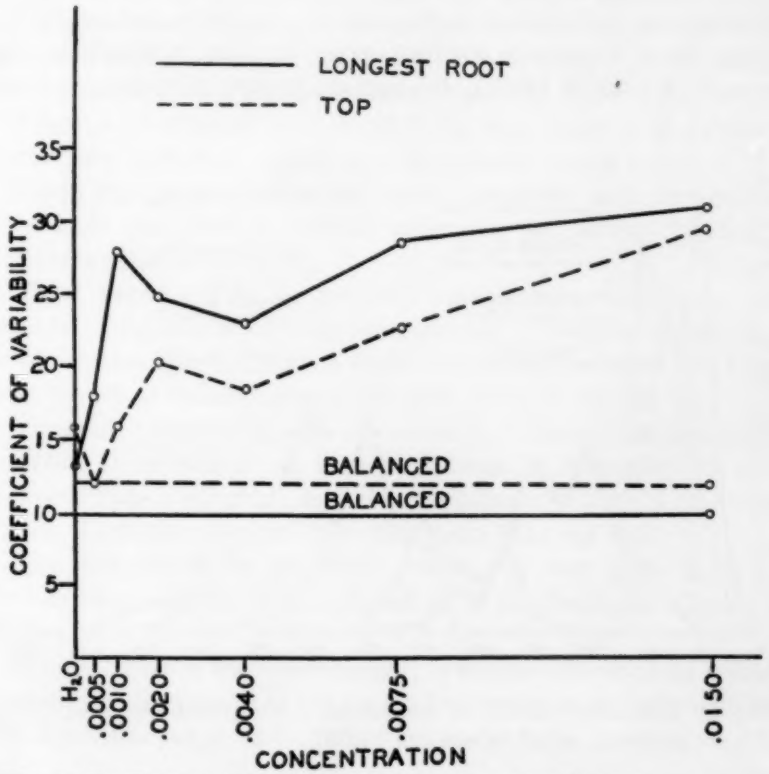

Fig. 2. Coefficients of variability for longest roots and tops.

ditions for the wheat seedlings (water, only in earlier stages of seedling growth). The variability is larger in the magnesium sulphate solutions, it rises in the lowest concentrations for tops as well as roots, falls for both of them at the concentration of 0.0020 M and 0.0040 M, and then rises again for roots as well as tops in the highest concentrations tried. This confirms the previous work of Goldschmidt (1928), Jennings (1908), Klebs (1907), Bumpus (1898), Duncker (1904), and others, indicating that the variability of organisms increases in changed conditions. The variability becomes greater in our case with the increase in the concentration of mag-

nesium sulphate. Variability coefficients are larger for longest roots than for tops.

Figure 3 shows the influence of the different solutions upon the variability curves of the longest root. The skewness figured by Pearson's formula was very slight. For the longest roots it was 0.1 mm. in distilled water, 0.4 mm. in 0.0005 M $MgSO_4$, 0.4 mm. in 0.0010 M $MgSO_4$, 0.4 mm. in 0.0020 M $MgSO_4$, 0.6 mm. in 0.0040 M $MgSO_4$, 0.1 mm. in 0.0075 M $MgSO_4$, 0.3 mm. in 0.0150 M $MgSO_4$, and 0.1 mm. in the balanced solution. For tops it was 0.1 mm. in distilled water, 0.6 mm. in 0.0005 M $MgSO_4$, 0.1 mm. in 0.0010 M $MgSO_4$, 0.8 mm. in 0.0020 M $MgSO_4$, 0.5 mm. in

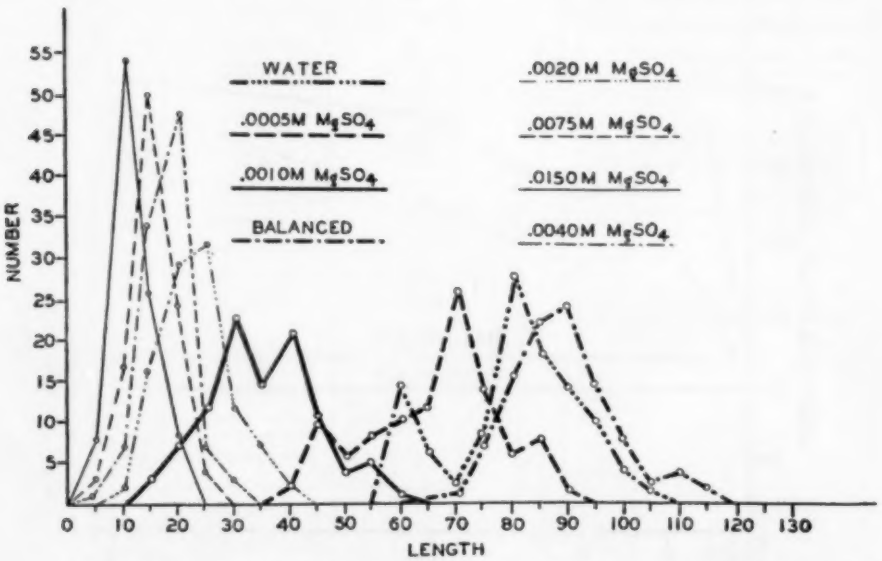

Fig. 3. Variability curves for longest roots. Ordinates represent number of individuals; abscissas, length in mm.

0.0040 M $MgSO_4$, 0.6 mm. in 0.0075 M $MgSO_4$, 0.4 mm. in 0.0150 M $MgSO_4$, and 0.5 mm. in the balanced solution. It can clearly be seen that the variability curves move to the left with the increase of the concentration. Simultaneously there occurs a gradual decrease of the dispersion range and a rise of the mode. Distilled water and the balanced solution show approximately the same variability curves as the lowest concentrations of magnesium sulphate, they are moved only slightly to the right. The same thing is brought out by the measurements of the other organs (principal root, left lateral root, right lateral root, and top). The jointed tops of the modes of the variability curves form a smoothly running curve, similar for all measured organs of the plant.

SUMMARY

A study was made of growth and variability of very young wheat seedlings in a wide range of concentrations of magnesium sulphate solutions. One hundred seedlings growing in the dark were tested with each solution, and the elongation of principal roots, left lateral roots, right lateral roots and tops was studied. The main results follow:

1. Growth of roots as well as of tops, when plotted against concentration of magnesium sulphate, gave smooth curves that fall at first rapidly in the lower concentrations tested, then with decreasing rapidity; in highest concentrations tested the curves become parallel to the base.

2. Magnesium sulphate was found to be more toxic to roots than to tops, and more toxic to principal roots than to the lateral roots.

3. Roots grew in distilled water almost as rapidly as in the balanced solution, while tops grew in distilled water only half as rapidly as in the balanced solution.

4. There was no correlation whatever between measured organs in any of the tested magnesium sulphate solutions nor in distilled water. In the balanced solution there existed a slight correlation between the longest root and the top as well as between the principal root and the top.

5. Variability coefficients were lowest in the balanced solution and distilled water; their values rose with an increase in concentration of the magnesium sulphate solution, with the exception of 0.0020 M and 0.0040 M $MgSO_4$. They were larger for longest roots than for tops.

6. The skewness of the variability curves was very slight in all magnesium sulphate solutions tested as well as in the balanced solution and distilled water.

7. With increase in the concentration of magnesium sulphate solutions there occurred a movement of the variability curves to the left, a decrease in their dispersion range, and a rise of the mode.

This opportunity is gladly taken to express indebtedness to Dr. Sam F. Trelease for suggesting the problem of the present work as well as for directing and criticizing it; to Mrs. Helen M. Trelease for valuable suggestions on the technique; and to Dr. Scheinkin for valuable help in calculating and computing the results as well as for criticizing the entire work.

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INDEX TO AMERICAN BOTANICAL LITERATURE
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